

SOCIETY

# - Grassland a European Resource?

Edited by Piotr Goliński Marianna Warda Piotr Stypiński



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## **Grassland – a European Resource?**

Proceedings of the 24<sup>th</sup> General Meeting of the European Grassland Federation Lublin, Poland 3–7 June 2012

> *Edited by* Piotr Goliński Marianna Warda Piotr Stypiński

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### Foreword

The main theme of the 24<sup>th</sup> General Meeting of the European Grassland Federation is 'Grassland – a European Resource?'. It is well known among grassland experts that grasslands are important resource in agriculture, environment and landscape. A question however arises whether grasslands are really perceived in Europe as important resources. Forages obtained from grasslands are often supplemented by maize, number of grazing animals has decreased in recent decades, previously utilized meadows and pastures have been abandoned in many regions and lost their high biodiversity.

The main topic of the 24th General Meeting of the EGF aims to stimulate discussion about the future of European grassland management and multi-functionality. The subject is 'hot' and of great importance but it is not completely new because for the last two decades multi-functionality of grassland has been a key concept of European agriculture and one of the fundamental directions of EGF policy. Grasslands are treated not only as an important source of fodder for animals which provide energy and proteins required for milk and meat production but also as a major element of the European landscapes, habitats for many plants and animal species, source of biodiversity, place of human activity. European society expects permanent grasslands which guarantee safety food and natural biodiversity but also are important in the aspect of cultural and social image. We are sure that grassland ecosystems are valuable European resources but, on the other hand, they are under threat and considerable pressure and their stability is in danger. It is our duty to protect the existing grasslands and secure their multi-functions for future generations but it needs more collaboration between scientists from different disciplines and requires new bridges between science, practice and agricultural policy. This was the background for us to select the main theme of the 24<sup>th</sup> General Meeting of the EGF in Poland.

Five plenary sessions have been prepared; each session is introduced by two plenary papers given by very well-known experts. We would like to thank all of them for accepting our invitation and excellent work. We have received many contributions which provide interesting, new ideas and promising approaches. After final reviews, 206 papers have been published as full scientific papers and 46 as abstracts. We would like to thank many people who have contributed to the conference. We express our gratitude to 300 delegates from more than 30 countries all over the world for their activity in preparing lectures, papers, posters and workshops. We thank numerous people for their help and effort made in order to organize the meeting: members of the Organizing and Scientific Committees and external reviewers for their hard work. Special thanks should be given to Mariusz Kulik, the secretary of our Meeting for his tremendous work, Roger Wilkins and Josef Nösberger - Honorary Life Presidents of EGF - for consultations of invited papers and also Alan Hopkins for the English language revision of all contributions. The Executive Committee of EGF was also very helpful in preparing our conference. Our very special thanks go to the EGF Secretary Willy Kessler for his advice and help. The conference would have been difficult to organize without the financial support of sponsors from industry and public bodies. We hope that the conference in Lublin will be an excellent occasion for up-to date research and will become a forum for open discussion between scientists, advisory people and farmers.

Fifteen years ago Poland was a host of the EGF Occasional Symposium. Now we have a great honor and pleasure to offer our hospitality and facilities to all participants of the General Meeting.

Have the most fruitful and enjoyable scientific and social program.

Piotr Goliński	Piotr Stypiński	Wanda Harkot
Chairman	President of the EGF	Chairman
of the Scientific Committee		of the Organizing Committee

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# **Opening session**

#### **Grassland** – a Polish resource

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#### Abstract

Permanent grasslands cover about 10% of Poland's territory and 21% of agricultural land. Meadows represent 77% and pastures about 23% of the grassland area. The peculiar characteristics of Polish grassland are their persistency, various conditions of their habitats, high floristic diversity, multifunctionality expressed in the predominance of mowing over grazing, moderate and low intensity of use and also very important roles in the natural environment, culture and landscape. This paper describes some results from grassland research and discusses grassland management in relation to the spatial distribution of grassland in Poland and factors determining their occurrence and specific features. The challenge for the future of grassland in Poland is to integrate scientific results and management practices to provide productivity and quality as well as utilitarian functions for the environment and at the land-scape level. Fodder production on grasslands as well as maintenance of grasslands to protect biodiversity, environment resources and landscape values need taking relevant legal measures and financial support ensuring decent living standards and working conditions to farmers.

Keywords: grassland management, spatial distribution, floristic diversity, multifunctionality

#### Polish grassland

The 24<sup>th</sup> General Meeting of the European Grassland Federation will take place in Poland, a country with a glorious but also difficult and dramatic history. This description also applies to grassland sciences and grassland management. Poland boasts a recorded history of grassland management over a millennium (Falkowski and Karłowska, 1961). Although this monograph is not as exhaustive as Franklin's (1953) work on Great Britain, it has presented an incentive for further research. The study by Falkowski and Karłowska (1961) contains information on the area of meadows and reclaimed land. It also follows a certain regional approach characteristic of more modern times. We are convinced that one cannot act for the future without knowing the past.

The history of grassland management over a millennium is undoubtedly very unique and different from the histories of other European countries. Several stages can be distinguished here: 10th-13th century – appreciation of meadows as a source of fodder,

14th-18th century – competition between cereal crops and meadows,

18th-19th century – subjugation characterised by contrasts: stagnation as well as a revival of grassland management,

20th century (up to 1939) – period of regained independence: a boom in the development of grassland management and grassland sciences,

1945-1989 - communist stagnation and suspension of the laws of market economy,

starting from 1989 – a turning point in Poland's political history and the beginning of the path of economic development according to the laws of market economy.

#### The area and distribution of permanent grasslands

Poland is a country with varied topographic, climatic and edaphic conditions. This diversity has multiple consequences manifested primarily in land use. According to data from 2010, utilised agricultural area (UAA) (with varying soil quality) occupies an area of 15.5 million ha, i.e. about 51% of Poland's territory. The area of forests amounts to 9.3 million ha (about 30%). Permanent grassland covers an area of 3.255 million ha, which accounts for 10% of Poland's territory and about 21% of agricultural land (National Agricultural Census, 2010). In Europe, permanent grasslands cover about 8% of the total area, but they account for 35% of UAA. The share of permanent grassland in the total area of Poland is considerably smaller than in many other European countries. The highest proportions of grasslands are found in certain countries in the northern and western part of Europe (Iceland, Norway, United Kingdom, the Netherlands, Switzerland, Austria). Countries with a lower grassland area than Poland include Denmark, Sweden and Hungary (Smit *et al.*, 2008).

In recent years, the proportion of UAA in Poland has decreased from 16.9 to 15.5 million ha. Arable land is the most dominant as it accounts for 68% of the total area of UAA. Within the sown land, the area of cereal, potato and sugar beet crops has declined, and the area of rapeseed and forage crops has increased. In comparison with the state of grasslands in 2002, the area of permanent meadows increased (Table 1) but the area of permanent pastures has fallen by almost 40%.

Permanent grassland	Years				
	1996	2000	2002	2005	2010
Area, million ha	4.13	3.85	3.56	3.39	3.25
Percent of UAA	23.1	21.9	21.1	21.3	20.9
including:	14.0	14.2	15.0	15.0	175
meadows	14.8	14.2	15.0	15.9	17.5
pastures	8.3	7.7	6.1	5.4	3.4

Table 1. Changes in the area and use structure of grassland in Poland (GUS, 2011)

The pattern of land use reflects the size and structure of farms. More than 96% of farms are individually- and family-owned, and in about 70% of these, the average area of farm does not exceed 7 ha. In the years 2002 to 2010, the structure of agricultural farms was slowly changing. In 2010, in comparison with 2002, the number of farms fell by 22% but their average area grew (National Agricultural Census, 2010), which enhanced their competitiveness and economic efficiency (Cieślik and Żmija, 2010). The farms grew through the purchase or lease of land. However, regional differences in the structure of farms remain. South-eastern Poland is characterised by a large number of small farms, while farms with the largest tracts of land can be found in northern Poland.

The predominance of small farms is linked with a large number of people employed in Polish agriculture. In 2008 they accounted for 14% of the total number of employed people. 74% of individuals in that group worked exclusively on their own farm, while work outside their farm was the main source of income for about 24%. The level of employment in Polish agriculture was 12.2 individuals per 100 ha of agricultural land, while in the leading EU member states it was 4 individuals per 100 ha.

As regards grasslands in Poland, meadows and pastures predominate in the provinces of north-eastern and south-eastern Poland (Podlaskie, Warmińsko-Mazurskie, Małopolskie and Podkarpackie – Figure 1). In the Lubelskie voivodship, meadows and pastures cover 228 thousand ha, which accounts for about 16% of UAA.



Figure 1. Permanent grassland in utilised agricultural area, livestock (100 ha<sup>-1</sup> UR) and % of ensilaged crops in Poland in particular voivodships (acc. to Jankowska-Huflejt *et al.*, 2011)

At the national level, meadows represent 77% and pastures about 23% of the total grassland area in Poland. Hay production from permanent meadows was over 12.1 million tonnes, and green fodder production from permanent pastures (converted into air-dry matter) amounted to about 2.9 million tonnes. The average yield of hay from permanent grassland was over 4.7 t ha<sup>-1</sup>.

The persistence of grass ecosystems depends on ensuring the biomass produced there is harvested. If the grasslands are not cut or grazed their composition will change and in many cases there will be development of woody species. In 2009, the area of non-utilised grasslands in Poland amounted to: in the 1<sup>st</sup> cut – 16.4%, in the 2<sup>nd</sup> cut – 17%, in the 3<sup>rd</sup> cut – 28.2% of permanent meadows (GUS, 2011).

#### The determinants of grassland occurrence

Water is the main factor determining the properties of grassland habitats and phytocenoses in Poland (Grzyb and Prończuk, 1995). Meadows and pastures occur in river valleys and mid-field depressions as well as in foothill and mountain areas having higher precipitation amounts than in the rest of the country. Grasses are dominant among the plants forming grassland communities, and their high water demand is a factor influencing the distribution of permanent grasslands in Poland's countryside.

Achieving sufficient humidity of grassland habitats depends on adequate water management, particularly with regard to maintaining the operational water and drainage facilities and restoring dysfunctional systems. Action is often required to ensure the collection of any amount of water (small water retention) with it being important to retain water from the catchments of particular rivers in early spring (Nyc and Pokładek, 1998; 2008).

The largest proportion of Polish grasslands is in valley meadows, which comprise: dryground meadows, flooded meadows, boggy meadows and post-boggy meadows. Boggy meadows occur in wet habitats and they are not important for fodder production, but they play an important environmental role. The other types of meadows are used for fodder production. However, insufficient soil humidity levels, resulting from small amounts of precipitation, lead to decreased persistence and productivity of these meadows, particularly in dry-ground habitats (Baryła and Kern, 1987). Insufficient humidity levels in post-boggy meadows lead to the degradation of peat-muck soils occurring there and, in consequence, to unfavourable changes in the floristic composition of these phytocenoses (Kiryluk, 2008; Kowalczyk and Łękawska, 1977). The optimum environmental and economic effects in valley grasslands (particularly located on organogenic soils) can be achieved by using surface irrigation and upward-irrigation systems (Nyc and Pokładek, 2008).

Thus effective water management in a habitat is the basic condition required to enable further measures aimed at improving the production from permanent grasslands through fertilisation, management and technology.

#### The peculiar characteristics of Polish grassland

Nearly all Polish grasslands occurring in their typical habitats are permanent ecosystems. Most of these grasslands have been characterised by great biological diversity both in the past and today. This results from the influence of favourable natural conditions (location in a central part of the continent, in a transitional climate zone, lack of natural barriers in the east and west, varied geological structure and diverse landforms) as well as from the peculiar impact of human activity, different than in other European countries (uneven degree of industrialisation and urbanisation, low-input farming with a moderate level of fertilisation and land use, as well as extensive and historically permanent forests).

The biological diversity has also been influenced by environmental protection measures that have had a long tradition in Poland (Fijałkowski, 2003). The protection mechanisms (i.e. creation of landscape parks, remaining natural forests and other ecosystems) implemented in order to preserve the biological diversity have resulted in the wealth of plant species and communities in Polish landscape (Olaczek, 1998).

Biodiversity may be very high in permanent meadows. More than 700 species of vascular plants and their presence in 100 different botanical families has been found in grassland communities (Kozłowski and Stypiński, 1997, according to Jasnowski). Some of them are very rare in Europe and they are protected by law. Polish meadows are characterised by the floristic diversity of their sward, which distinguishes them from meadows in other countries, particularly in Western Europe. The results obtained by Kryszak (2001) concerning the Wielkopolska region can be regarded as characteristic. The main factors determining the floristic diversity of a grassland sward are the properties of grassland habitat and the intensity of farming practice and utilisation (Kostuch, 1995; Mosek and Miazga, 2006; Wyłupek, 2002). The results of studies conducted by Kryszak *et.al.* (2011) show that the values of the Shannon-Wiener indicator (H'), characteristic of meadow associations in the mountains and foothills are higher than for meadows in the lowlands. More sward species are also recorded in the plant communities of permanent grasslands occurring on mineral soils than on organic soils (Baryła, 2001; Gajda, 1987).

A proper analysis of the floristic diversity of meadow communities must take into account their syntaxonomy. The floristic diversity of the same associations and communities may vary across different regions of Poland (Kucharski, 1999). Typical associations, occurring in the optimum habitat conditions, are characterised by a greater floristic diversity than variants with a clear domination of one species. Similarly, communities in very wet habitats (the *Phragmitetea* class) are characterised by a smaller species diversity resulting from

a small number of dominant species (Kryszak and Grynia, 2005). A much greater floristic diversity characterises the phytocenoses of moderately wet and periodically dry habitats of the *Molinio-Arrhenatheretea* class (Szoszkiewicz and Szoszkiewicz, 1999; Warda and Stamirowska-Krzaczek, 2010).

Changes in habitat conditions and varying intensity of utilisation lead to the disappearance of many plant species. Under conditions of increasing fertilisation, the number of species in the sward declines, particularly in the case of increased nitrogen and phosphorus content in the soil (Kryszak, 2001). In recent years, some meadow and pasture areas have ceased to be utilised (Trąba *et al.*, 2006). This abandonment of farming practices also decreases the biological diversity of grassland, particularly in less fertile habitats and at higher altitudes (Dradrach *et al.*, 2007; Kasperczyk and Szewczyk, 1999; Zarzycki and Misztal, 2010).

An increased interest in grassland management around the world could be observed as early as the turn of the 20th century, but a period of intensified agricultural production occurred in the second half of the 1900s. A measurable effect of this intensified production was an increase in the fodder produced and higher animal production rates, whereas the side effects included changes occurring in habitats and phytocenoses manifested, for example, in decreased biodiversity and persistence of ecosystems.

The productivity of grasslands in Poland in that period was generally lower than in the "old" EU member states (EU15) although quite a high productivity of permanent grasslands was achieved in the 1970s and 1980s (Grzegorczyk, 2010).

At that time, wet habitats in Poland were drained and reclaimed for farming, mainly using the total cultivation method (Gajda, 1974). The sward of the newly established grasslands, consisting of valuable fodder grasses, was characterised by greater productivity (Prokopowicz *et al.*, 2008) but was less varied floristically, and the use of more regular and increased mineral fertilisation, particularly nitrogen, led to a low number of species in the sward (Baryła, 1992; Doboszyński, 1973; Mikołajczak, 1996). Although relatively intensive fertilisation took place on large and specialist farms, the average consumption of mineral fertilisers per 1 ha of UAA in Poland was small as it amounted to 86 kg in the farming year 1999/2000, about 123 kg in 2005/2006, and about 122 kg NPK in 2009/2010. Average fertiliser applications to grassland was lower than for UAA but precise figures are not available. The regional variation in the amount of fertiliser applied has also been confirmed. The highest levels of average fertiliser consumption were recorded in the province of Opolskie (187 kg ha<sup>-1</sup>), with Kujawsko-Pomorskie (175.5), Dolnośląskie (159) and Wielkopolskie (155) also having high application rates (National Agricultural Census, 2010).

At the same time, the intensification measures had a favourable influence on the efficiency of agricultural production, but a negative impact on the natural environment (Prokopowicz and Kowalczyk, 2007). The results of long-term studies (1963–1988) on valley grasslands indicate a high level of productivity (8–10 t DM ha<sup>-1</sup>) in the first years after draining the peat bogs and farming them using the ploughing and resowing. However, as the utilisation of the grasslands continued, the yield and quality of hay systematically decreased (Gotkiewicz and Szuniewicz, 1968) and the natural fertility of the soil deteriorated in due to the progressive mineralisation of organic matter

Furthermore, the frequency of meadow utilisation and the stocking rate of animals increased in that period. However, these measures did not always produce the desired results in animal production due to the low genetic merit of the animals used (Żurek, 2001).

In regions with a limited area of grasslands and a predominance of arable land, temporary grasslands play an important role in the production of bulky fodder. Grass-legume mixtures are particularly important among plants cultivated on such grasslands because their production potential and high nutritional value ensure that the feeding requirements of high-production ruminants are met (Harasim, 2008; Kryszak and Kruczyńska, 1998).

#### Multifunctionality of grassland in Poland

#### Fodder utilisation of grassland

The production of fodder for animals has been the main goal of meadow and pasture utilisation so far (Gajda and Warda, 1987; Goliński, 1995a; Okularczyk, 2001). The production of such fodder should take into account the main objectives of sustainable agriculture, particularly ensuring decent living standards to farmers, adequate care for the health of people and animals, environmental protection, biodiversity, preservation of cultural values and cultural heritage of rural areas (Grzegorczyk, 2010; Wasilewski, 2009).

The primary use of Polish grasslands is cutting in order to obtain hay (over 60% of the first and about 50% of the second cutting) and, to a smaller extent, to produce silage (15%). Comparing various regions, the largest amount of silage from meadow swards (30%) is produced in the Podlaskie province (Jankowska-Huflejt and Domański, 2008).

Fodder from grasslands is very important in the nutrition of ruminants (Gajda *et al.*,1994; Goliński, 1995b; Gruszecki *et al.*, 2001; Lipińska and Gajda, 2006; Litwińczuk *et al.*, 2001; Rogalski *et al.*, 1998; Warda, 1996; Warda and Krzywiec, 2001; Wasilewski, 2011). In countries where permanent grasslands predominate, it has been found that the area of meadows and pastures is correlated with the number of cattle and volume of dairy production achieved with fodder obtained from grasslands, even though this correlation varies strongly across the particular regions in Europe (Smit *et al.*, 2008). Similar trends can also be observed in Poland in large-area farms with a large proportion of grasslands, where pasturing is a rational method of feeding dairy cows and, to some extent, beef cattle. However, cattle are not always put to pasture. The pasturing of dairy cattle has decreased not because of the low effectiveness of this feeding method, but in order to limit the involvement of the labour force, particularly in farms with a small number of staff (Wasilewski, 2011).

There is a close relationship between the use of grassland and the livestock. This is evidenced by a decline in the numbers of farm animals since 1980 (Table 2). There was a massive reduction in the number of cattle from 1990 to 2000, but from 2002 to 2010, the number of cattle in Poland increased by 4% but dairy cows declined by about 8%, which resulted from a decrease in the number of small farms (up to 2 ha) with cattle as well as from restrictions imposed after Poland's accession to the European Union (requirements concerning cowshed equipment, the quality of milk purchased from farmers, and individual milk quotas). Pasturing is continued on medium-size farms specialising in the breeding of dairy cows. In 2010, the average annual milk yield was 4487 litres per cow. The average milk yield in specialist dairy farms is considerably higher (Dembek and Łyszczarz, 2008; Wasilewski, 2011). The number of cattle also varies across regions. With regard to production related to cattle (size of herds, production of milk and beef livestock), three provinces are in the lead: Mazowieckie, Podlaskie and Wielkopolskie.

Farm animals	Years				
	1980	1990	2000	2002	2010
Cattle	12649	10049	6083	5533	5755
Sheep	4207	4159	362	345	268
Horses	1780	941	550	330	264

Table 2. The number of farm animals (thousand heads) in Poland (GUS, 2011)

#### Other uses of grassland

Obtaining fodder from swards is the most natural and basic form of using permanent grasslands in Poland and other European countries. However, sometimes the sward obtained cannot be used as fodder and has to be used for other purposes. This has particularly been the case in Poland following the large reduction in the number of farm animals. There has been a large increase in the use of meadow swards as energy biomass. Its key advantages include a low content of non-flammable ingredients, low sulphur and nitrogen oxide content, and zero balance  $CO_2$  emission. The heating value of hay is put at 16–17 MJ kg<sup>-1</sup>, whereas the estimated heating value of coal is 25 MJ, and that of natural gas is 50 MJ. The heating value of sward is determined by a number of factors, including its floristic composition (Harkot *et al.*, 2007).

Meadow swards with the predominance of *Phalaris arundinacea* enjoy considerable popularity. However, a paradoxical situation exists in Poland. In the past, *Phalaridetum arundinaceae* meadows were widely appreciated for the high production and quality of the sward, but at present they are valued for the high yield and heating value of the sward (Księżak and Faber, 2007). At the same time, the area of *Phalaridetum arundinaceae* meadows is shrinking. In the ongoing debate on whether existing meadow swards or phytomass from special grass crops such as the *Miscanthus* grasses is a better source of energy biomass, economic factors are in favour of meadows.

Another challenge is the possibility to obtain biogas from meadow swards. Currently it may be concluded that it is more appropriate to use swards from leys, particularly those with predominance of *Lolium multiflorum*. According to Goliński and Jokś (2007), the efficiency of biogas from these crops is nearly 50% greater than in the case of *Phalaris arundinacea* crops (3000 m<sup>3</sup> of biogas per ha).

Meadow swards or biomass from sown grass species and cultivars could be a source of cellulose pulp for paper production. However, there has so far been no interest in developing this application. The silvopastoral system has not become popular in Poland either, both in cognitive and utilitarian terms.

Permanent grassland is often viewed as a source of nectar for bees (*Apidae*) since the herbrich swards attract these insects. However, there are large differences in the attractiveness of the individual herb species (Kaczmarek, 2009). It was shown that within one minute in the summer, the flowers of the blooming sweet clover (*Melilotus*) are pollinated by 5 honey bees, white clover (*Trifolium repens*) - by 4 bees, birdsfoot trefoil (*Lotus corniculatus*) – by 2.5 bees, and meadow vetchling (*Lathyrus pratensis*) – by 0.87 bees. A clear advantage of a meadow is a long blooming season of the individual elements of the sward. Meadow swards are also a valuable source of pollen but there is no figures on the economic importance of nectar and pollen from grasslands. There are a large number of meadow herb species, and the presence of the individual taxa is determined by the humidity of the meadow habitat (Wilkaniec *et al.*, 1996; Kołtowski, 2006).

Permanent meadows have long been valued for the medicinal properties of herbs used in the treatment of people and animals. The works of Krzysztof Kluk, who lived in the late 18th and early 19th century, are an important source of information in this respect. The list of medicinal species has been amended with increased understanding of the chemical properties of herbs and the meadow habitats suitable for the growth and development of some specific taxa have been destroyed. Rychnovska's opinion (1994) concerning the role of meadow herbs as an authentic source of pharmacological material remains valid. However, herbs from grasslands or medicinal pharmaceuticals extracted from them are not used commercially.

#### Environmental functions of grassland

It is well known that permanent meadows are located in special habitats formed by soils of organic origin. As evidenced by Grzyb (1967), the layer of well-decomposed peat, 1 metre deep, has a water capacity of 7500 m<sup>3</sup> ha<sup>-1</sup>. The same layer of peat-muck soil, on the other hand, absorbs between 4000 and 5500 m<sup>3</sup> of water, and sandy alluvial soil – at least 3500 m<sup>3</sup>.

Peat soils are also rich in nitrogen, the rate of release of which is determined by water relations. In meadows located in very humid soils, small amounts of nitrogen are released – about 65 kg within a year; in meadows with dry soil – 303 kg ha<sup>-1</sup>; and in ploughed meadows – as much as 346 kg ha<sup>-1</sup>.

The anti-erosion function of permanent meadows is another argument for their preservation even though the problem of erosion is not as acute in Poland as in other regions of the world. According to calculations, the soil layer may subside even by 2 cm per year in higher locations in the Carpathians and with high slope gradients (Lipski and Kostuch, 2005). For environmental reasons, grassland has an enormously important role as a biogeochemical barrier preventing the migration of biogenes from the soil to surface water (Kozłowski *et al.*, 2009). The role of meadows in this respect is corroborated by the results of studies on Poland's soil and climate conditions presented by Kopeć (1989). These results are still reliable and valid. A permanent meadow complex crossed even by a small river can cause a reduction of nitrate-nitrogen content from 49.9 mg to 2.8 mg l<sup>-1</sup>, and potassium content from 15.0 mg to 2.4 mg l<sup>-1</sup>.

The preservation of permanent meadows is also justified by their positive role in the shaping of the climate and microclimate. It has been calculated that soil temperature at the depth of 5 cm is 1.1°C higher under swards at the pasture growth stage than under meadows, but 3.8°C lower than in soil without vegetation. These differences also occur, albeit to a lesser extent, in the lower horizons of a soil profile (Kozłowski and Swędrzyński, 2010). Meadows are also a habitat for many animals species, primarily birds (Tomiałojć, 1990; Tomiałojć and Stawarczyk, 2003).

#### The role of meadows in the shaping of landscape and culture

Grasslands, both permanent and temporary, are an integral element of the Polish landscape determining its character and identity. A special role is played by large meadow complexes stretching along river valleys, particularly of such rivers as Noteć, Narew, Obra and Warta. These complexes are a result of drainage works carried out in order to reclaim the swamps existing there in the past. Until recently, the network of canals then created could only be seen from a bird's eye view, but this arrangement has been disrupted by a lack of meadow maintenance. The layout of the drainage ditches is now marked by spontaneously growing shrubs and trees, mainly black alder and various willow species. As a result, large meadow complexes appear to be more varied and have a thicker texture. In the north-eastern regions of Poland, meadow complexes are peppered with haystacks, even in the winter, forming an integral part of a typical Polish landscape (Marks and Nowicki, 2010).

Meadow complexes are also covered with carpets of blooming herbaceous plants, mainly herbs. The floristic composition of the sward determines the mosaic-like diversity of colours and emphasises the seasonally dominant colours. Thus, during the growing season, the same meadow undergoes colour changes, for example from white, resulting from the dominance of *Cardamine pratensis*, to yellow, an effect of various species of *Ranunculus*, to pink associated with the dominance of *Lychnis flos-cuculi*, and to blue, caused by the blooming of *Geranium pratense*. It should be added that the green of grasses constitutes a strong

background to the colour of herb flowers, which enhances the visual effect (Kozłowski, 2007). A similar role in the landscape is played by smaller meadows or even small meadow enclaves scattered amid the fields with various agricultural crops.

The visual aspect is somewhat disrupted by meadows where sward cutting has been abandoned or fields laid fallow and spontaneously colonised by various tree and shrub species. Unfortunately, these areas, not used for agricultural purposes, will have an increased share and a stronger presence in the landscape (Trąba, 2004).

Polish meadows, with their floristic diversity, have always been a rich source of aesthetic impressions. Therefore, they are part of the history of Polish culture: in the sphere of literature, painting, and even music. The list of references is very rich. It is worth mentioning a novel by Stanisław Vincenz from the turn of the 20th century, 'Na wysokiej połoninie' ('On a High Mountain Pasture'), containing a detailed description of meadow plants which enables an accurate identification of the species. It should also be added that a lot of poetry featuring meadow motifs is created nowadays. This shows that the beauty of meadows, appreciated by very few in the past, is now becoming a reality for many people sensitive to the beauty of nature.

As far as painting is concerned, one should mention the great work entitled 'Łąka' ('A Meadow') created by a Poznań painter Andrzej Okińczyc. Not only does the artist faithfully highlight the flora of a meadow, but he also masterfully conveys the rippling sward. The motif of the meadow was memorably introduced into Polish music by Zygmunt Noskowski (late 19th and early 20th century), particularly in his musical poem 'Step' ('The Steppe'). In order to achieve the sound effect, an artist has to demonstrate tremendous sensitivity as well as considerable knowledge of nature.

#### Grasslands: a natural resource that has to be preserved

Given the multiple functions of meadows and their positive role in Polish landscape and culture, as described above, one can make a legitimate claim that grasslands constitute a natural resource which should be rationally used and, above all, preserved for the future generations. Multi-faceted actions of various decision-making bodies and, most of all, greater social awareness are required in order to achieve that goal.

The preservation of meadows depends on their moderate fertilisation, rational utilisation and proper maintenance, and that particularly includes the defoliation of their sward (Kulik, 2010). This is a key measure preventing the spontaneous colonisation of meadows by trees and shrubs. There is no doubt that the future of meadows as sources of fodder requires their owners and users to be familiar with the rational utilisation and maintenance of meadows, as well as be aware of the multiple functions of meadows. That knowledge should be supported by expert assistance from agricultural advisers. The legally sanctioned ownership does not exempt the owners of meadows from responsibility for their future.

Owning meadows surely brings benefits to their owners. The future of meadows also necessitates legal measures that will ensure the profitability of dairy and beef production based on the fodder from meadow complexes. Such measures are indispensable for preventing meadows from being degraded, ploughed over and converted into arable land.

The knowledge of our society about the role of meadows in agriculture and their significance for the natural environment and landscape varies considerably, and generally, there is a lot of room for improvement. However, there is a growing understanding that meadows, similarly to forests, constitute a natural resource for the entire nation. Such an attitude is conducive to educational, research and legal measures. The preservation of meadows also requires assistance from the state. The significance of meadows in various areas is more and more appreciated by the Polish state authorities that collaborate with other EU member states in the implementation of the Common Agricultural Policy and of the Natura 2000 network. Agricultural policy of the European countries develops in two directions: in the area of agricultural production, particularly with regard to the market and prices as well as direct subsidies, and in the area of supporting agro-environmental measures. Thus issues related to meadow-management are part of the state policy. This particularly concerns the floristic diversity and biodiversity of ecosystems.

The Natura 2000 programme, developed pursuant to the Birds Directive and Habitat Directive, also deserves special appreciation. "Maintaining extensive meadows" is worthy of note among environmental packages. It determines the future of Polish meadow bogs, wet and stenothermic meadows, cut once or twice a year.

The European Commission has already approved 823 "habitat areas" in Poland, accounting for about 11% of the territory of Poland. Ultimately, "habitat areas" and "bird areas" are due to cover about 22% of Poland's area. These "areas" include the valleys of some rivers along with meadow complexes. The most important include the valley of the Barycz river, covering about 55500 ha, dry-ground and riparian forests in the Odra valley, covering about 38000 ha. Meadows in the landscape and national parks are also worth paying attention to. Currently, there are 23 national parks in Poland. Large meadow areas are located in the Biebrza National Park, the Narew National Park and the Warta River Mouth National Park. As for landscape parks, there are 120 parks at present, but their number is increasing. The meadows within some national and landscape park areas serve to actively protect nature through extensive grazing strategy (Nowakowski et al., 2008), frequently using the animals of local breeds (Warda et al., 2011). A limited cultivation level, in particular fertilization practices, proves conducive to maintain permanent grasslands and conservation of their floristic biodiversity (Sienkiewicz-Paderewska and Stypiński, 2009; Warda et al., 2011). Besides, great importance should be attached to a fact of grasslands reinstitution in the stands from which they were eliminated in the close and distant past.

#### Conclusions

Forward thinking imposes putting a question concerning the fate of the Polish grassland resources as well as future management practices. Undoubtedly, the area of permanent grasslands will not reduce drastically but importantly, their environmental function as well as role in the maintenance of the landscape will consistently increase. Whereas their contribution to animal feed production will depend on the market size for milk and meat. The market will determine the directions of animal production and the profitability of milk and meat production based on grassland forage feeds. With passing time, functional regionalism in grassland management will increase. The north-eastern part of Poland will become the dairy milk land. Beef production will also develop in other regions of Poland with farms having higher proportion of grasslands in UAA. Permanent grasslands will make pastures giving moderate sward yield obtained under the rational utilization and management practices but primarily, rational fertilization regimes. Implementation of such strategies will favour grassland persistence so as to leave the grasslands unimpaired for future generations. However, the use of swards for other non-fodder purposes will certainly progress, but to a tolerable extent. We do believe that public awareness and state policy (in the science domain, too) will support the maintenance and conservation of grasslands as a contribution to the natural wealth of our country.

#### References

Baryła R. (1992) Variability of meadows yielding on the organic soils under the conditions of a differentiated nitrogen fertilization, lasting for many years. *Wiadomości Instytutu Melioracji i Użytków Zielonych* 17, 2, 297–307.

Baryła R. (2001) Changes of the species composition of meadow undergrowth in post-boggy habitat (a synthesis of 30-year-long studies conducted in Sosnowica, the Wieprz-Krzna Canal Region). *Annales UMCS* E, 56, 65–79.

Baryła R. and Kern H. (1987) Yield of dry meadows depending on the differentiated mineral fertilization. Zeszyty Problemowe Postępów Nauk Rolniczych 308, 177–183.

Cieślik J. and Żmija J. (2010) Economic situation of dairy farms in Małopolska district. *Roczniki Nauk Rolniczych* G, 97, 3, 43–50.

Dembek R. and Łyszczarz R. (2008) Production potential and dietary value of fodder made from the Notec Canal valley grasslands. *Pamiętnik Puławski* 147, 31–43.

Doboszyński L. (1973) The impact of nitrogen fertilisation on the botanical composition of grasslands. Zeszyty Problemowe Postępów Nauk Rolniczych 150, 43–60.

Dradrach A., Gawęcki J., Gierula A., Sokolska D. and Wolski K. (2007) The floristic changes in resting greenlands in Sudety Mountains on the base of Łężyce near Duszniki. *Problemy Zagospodarowania Ziem Górskich* 54, 149–156.

Falkowski M. and Karłowska G. (1961) The development of meadow-pasture management in the Great Poland. *Prace Komisji Nauk Rolniczych i Komisji Nauk Leśnych Poznańskiego Towarzystwa Przyjaciół Nauk* 9, 2, 3–19.

Fijałkowski D. (2003) *Environmental protection in the Lublin region*. Lubelskie Towarzystwo Naukowe, Lublin, pp. 409.

Franklin T.B. (1953) *British grasslands from the earliest times to the present day*. Faber and Faber, 24 Russel Square, London, pp. 173.

Gajda J. (1974) The effects of the development of grasslands in the Parczew-Włodawa sub-region of the Wieprz-Krzna Canal. *Annales UMCS* E, 28/29, 229–300.

Gajda J. (1987) Non-flooded meadows in river valleys on the example of the Bug and Wieprz. Zeszyty Problemowe Postępów Nauk Rolniczych 308, 53–73.

Gajda J. and Warda M. (1987) Grasslands as the basis for the development of sheep breeding in the Biała Podlaska province. *Owczarstwo* 9 (123), 11–12.

Gajda J., Zalewski W. and Litwińczuk Z. (1994) Effect of pasture feeding on the fattening results of various beef cattle genotypes. *Roczniki Nauk Rolniczych* A, 110, 3–4, 181–192.

Goliński P. (1995a) Economical and technical conditioning of feed production on grasslands as dependent on the level of its intensity. *Biuletyn Oceny Odmian* 29, 11–25.

Goliński P. (1995b) Pasture as a privileged source of fodder on a farm: computer analyses. In: Trends in the development of grassland science in relation to the current state of knowledge in its key areas. *Materialy Ogólnopolskiej Konferencji Łąkarskiej*. Warszawa 27–28 września 1994 r. Wydawnictwo SGGW, Warszawa, pp. 157–164.

Goliński P. and Jokś W. (2007) Chemical and biological properties of grasses and biogas production. *Grassland Science in Poland* 10, 37–47.

Gotkiewicz and Szuniewicz (1968) Productivity of meadows on peat. Zeszyty Problemowe Postępów Nauk Rolniczych 83, 33–56.

Gruszecki T. Lipecka Cz., Lipiec A., Markiewicz J., Kamińska A. and Skałecka A. (2001) Evaluation of the carcasses and muscular tissue of lambs fattened on the pasture. *Zeszyty Problemowe Postępów Nauk Rolniczych* 479, 101–109.

Grzegorczyk S. (2010) Grassland in agricultural production in Poland against other countries of European Union. Zeszyty Problemowe Postępów Nauk Rolniczych 549, 61–69.

Grzyb S. (1967) Natural conditions of meadows and pastures productivity. In: *Wiadomości z ląkarstwa*, PWRiL, Warszawa, pp. 21–64.

Grzyb S. and Prończuk J. (1995) The classification and valuation of meadow habitats and assessment of their production potential. In: Trends in the development of grassland science in relation to the current state of knowledge in its key areas. *Materialy Ogólnopolskiej Konferencji Łąkarskiej*. Warszawa 27–28 września 1994 r. Wydawnictwo SGGW, Warszawa, pp. 51–63.

GUS (2011) Statistical yearbook of agriculture. Main Statistical Office, Warszawa, www.stat.gov.pl.
Harasim J. (2008) Yielding of pasture sward with white clover in relation to the seeding rates and habitat. *Water-Environment-Rural Areas* 8, 2b (24), 19–29.

Harkot W., Warda M., Sawicki J., Lipińska H., Wyłupek T., Czarnecki Z. and Kulik M. (2007) The possibility of meadow sward use for energy purposses. *Grassland Science in Poland* 10, 59–67.

Jankowska-Huflejt H. and Domański P. (2008) Present and possible directions of utilising permanent grasslands in Poland. *Water-Environment-Rural Areas* 8 (2b), 31–49.

Jankowska-Huflejt H., Wróbel B. and Twardy S. (2011) Current role of grassland in development of agriculture and rural development – an example of mountain voivodships małopolskie and podkarpackie. *Journal of Water and Land Development* 15, 3–18.

Kaczmarek Z. (2009) Floristic variability of permanent meadows in the aspect of food and habitat basis for bees. PhD thesis, Department of Grassland and Natural Landscape Sciences, Poznan University of Life Sciences, Poznań.

Kasperczyk M. and Szewczyk W. (1999) Sward floristic composition of mountain grasslands after cessation of use. *Folia Universitatis Agriculture Stetinensis* 197, *Agricultura* 75, 163–166.

Kiryluk A. (2008) The condition of drainage facilities and grassland productivity in Podlaskie province. *Water-Environment-Rural Areas* 8, 2b (24), 61–70.

Kołtowski Z. (2006) Great atlas melliferous plants. Przedsiębiorstwo Wydawnicze Rzeczypospolita SA, Warszawa.

Kopeć S. (1989) Le role des plantes herbagers dans la limitation des pertesdes pricipauxeleements nutritifs par le drainage. *Proceedings of the XVI International Grassland Congress*, Nice, 2, 1645–1646.

Kostuch R. (1995) The causes of the floristic diversity occurrence in the grassland ecosystems. *Annales UMCS* E, suppl., 50, 23–33.

Kowalczyk J. and Łękawska I. (1977) Characteristic of changes in the meadow sward and its productivity on distinguished sites of the Wizna fen. *Polish Ecological Studies* 3, 3, 55–65.

Kozłowski S. (2007) Grasses in the Polish landscape. In: *Księga polskich traw* (The Book of Polish Grasses), Frey L. (ed.), Institute of Botany, Polish Academy of Sciences, 389–411.

Kozłowski S. and Stypiński P. (1997) The grassland in Poland in the past, present and future. *Grassland Science in Europe* 2, 19–29.

Kozłowski S. and Swędrzyński A. (2010) Meadow – Environment – Human. Zeszyty Naukowe WSA w Łomży 46, 241–260.

Kozłowski S., Zielewicz W. and Swędrzyński A. (2009) Effect of permanent meadows on the chemistry waters. *Grassland Science in Europe* 14, 154–157.

Kryszak A. (2001) Floristic diversity of meadow and pasture associations of the *Molinio-Arrhenatheretea* R.Tx. class in Wielkopolska from the point of view of their economic value. *Roczniki Akademii Rolniczej* w *Poznaniu*, Rozprawy Naukowe, 314, pp. 182.

Kryszak A. and Grynia M. (2005) Grass communities of excessively wet sites in river valleys. *Grassland Science in Poland* 8, 97–106.

Kryszak A., Kryszak J. and Strychalska A. (2011) Natural and use value of meadow communities of mountain and lowland regions. *Grassland Science in Europe* 16, 490–492.

Kryszak J. and Kruczyńska H. (1998) Productivity and nutritive value of clover-grass mixtures cultivated on arable lands. *Zeszyty Problemowe Postępów Nauk Rolniczych* 462, 165–171.

Księżak J. and Faber A. (2007) Evaluation of possibility gain of biomass with red canarygrass on energy. *Grassland Science in Poland* 10, 141–148.

Kucharski L. (1999) The flora of the meadows of central Poland and its changes in the 20th century. Wydawnictwo Uniwersytetu Łódzkiego, Łódź, pp. 168.

Kulik M. (2010) Effect of cutting frequency of a meadow sward on the bulk density of a peat-muck soil. *Grassland Science in Europe* 15, 708–710.

Lipińska H. and Gajda J. (2006) Area of farms versus fodder base and cattle population in specialized dairy farms. *Annales UMCS* E, 61, 225–236.

Lipski Cz. and Kostuch R. (2005) Soil erosion processes in the Carpathian mountains Regions. *Infrastruktura i Ekologia Terenów Wiejskich* 3, 95–105.

Litwińczuk Z., Stanek P. and Jankowski P. (2001) Efficiency of pasture rearing of young beef bulls and heifers depending on the initial body weight at the beginning of pasture season. *Zeszyty Problemowe Postępów Nauk Rolniczych* 479, 193–202.

Marks M. and Nowicki J. (2010) Cultivated fields and green use areas in contemporary agricultural landscape. Acta Scientiarum Polonorum Administratio Locorum – UWM 9 (3), 95–106. Mikołajczak Z. (1996) Influence of mineral fertilization on quality and quantity of yields from Sudety mountains grasslands. *Zeszyty Problemowe Postępów Nauk Rolniczych* 442, 299–308.

Mikołajczak Z. and Warda M. (1997) Pasture productivity under limited mineral fertilization. Zeszyty Problemowe Postępów Nauk Rolniczych 453, 25–38.

Mosek B. and Miazga S. (2006) Floristic diversity of plant communities on reclaimed river valleys of Lubelszczyzna region. *Annales UMCS*, E, 61, 377–387.

Nowakowski P., Dobicki A. and Mikołajczak Z. (2008) The main feed of beef cattle extensively grazing in areas of a National Park "Warta Mouth". *Pamiętnik Pulawski* 147, 181–187.

Nyc K. and Pokładek R. (1998) The role of precipitation in decreasing water deficit under controlled runoff. *Water-Environment-Rural Areas* 1, 2 (2), 63–76.

Nyc K. and Pokładek R. (2008) The current problems of grassland drainage and reclamation. *Water-Environment-Rural Areas* 8, (2b), 97–103.

Okularczyk S. (2001) Economic efficiency of milk production based on grassland forage. *Pamiętnik Puławski* 125, 453–458.

Olaczek R. (1998). Biological diversity as the object of environmental protection. *Przyroda Polska* 9, 8–9. Prokopowicz J. and Kowalczyk J. (2007) Differences in yielding of meadows and pastures on peat soils in meadow experiments and in the production. *Water-Environment-Rural Areas* 7, 1 (19), 159–170.

Prokopowicz J., Jankowska-Huflejt H. and Burs W. (2008) Economical and ecological effects of meadow and pasture production on a long reclaimed object in the Por river valley. *Grassland Science in Europe* 12, 556–559.

Prusak A., Tabor S. and Murgaš J. (2009) The agricultural production balancing in the aspect of grassland resources and livestock. *Inżynieria Rolnicza* 6 (115), 217–222.

National Agricultural Census 2010. www.stat.gov.pl/gus/rolnictwo

Rogalski M., Kryszak J., Goliński P. and Binias J. (1998) Effectiveness of beef production from heifers under of extensive grazing conditions. *Grassland Science in Europe* 3, 255–259.

Rychnovska M., Blazkova D. and Hrabe F. (1994) Conservation and development of floristically diverse grasslands in Central Europe. *Proceedings of 15<sup>th</sup> General Meeting of the European Grassland Federation*, Wageningen, pp. 266–277.

Smit H.J., Metzger M.J. and Ewert F. (2008) Spatial distribution of grassland productivity and land use in Europe. *Agricultural Systems* 98, 208–219.

Sienkiewicz-Paderewska D. and Stypiński P. (2009) Permanent grassland in Polish landscape parks. Part II. The protection. *Grassland Science in Poland* 12, 177–187.

Szoszkiewicz K. and Szoszkiewicz J. (1999) Assessment of species diversity of selected meadow phytocoenoses łąkowych with different agricultural value. *Folia Universitatis Agriculture Stetinensis* 197, *Agricultura* 75, 307–312.

Tomiałojć L. (1990) Polish Birds. Distribution and Quantity. PWN, Warszawa.

Tomiałojć L. and Stawarczyk T. (2003) Avifauna of Poland. Distribution, abundance and changes. PTTP "Pro Natura", Wrocław.

Trąba Cz., Wolański P. and Oklejewicz K. (2004) Plant communities of abandoned meadows and fields in the San valley. *Grassland Science in Poland* 7, 207–238.

Traba Cz., Wolański P. and Oklejewicz K. (2006) Floristic diversity of selected non-forest communities of the San river valley. *Annales UMCS*, E, 61, 267–275.

Warda M. (1996) Productivity and persistency of grass-clover mixtures under cattle grazing. Zeszyty Problemowe Postępów Nauk Rolniczych 442, 441–451.

Warda M. and Krzywiec D. (2001) The effect of white clover on the pasture sward utilization by cattle. *Zeszyty Problemowe Postępów Nauk Rolniczych* 479, 273–277.

Warda M. and Stamirowska-Krzaczek E. (2010) Floristic diversity of chosen grass communities in the Nadwieprzański Landscape Park. *Annales UMCS* E, 65, 1, 97–102.

Warda M., Kulik M.A. and Gruszecki T. (2011) Characteristic of selected grass communities in the "Kózki" nature reserve and a test of their active protection through the grazing of sheep of the Świniarka race. *Annales UMCS* E, 1–8.

Wasilewski Z. (2009) Current status and directions of grassland management in accordance with the requirements of the common agricultural policy. *Water-Environment-Rural Areas* 9, 2 (26), 169–184.

Wasilewski Z. (2011) The efficiency of dairy cow grazing in a large farm. *Water-Environment-Rural Areas* 11, 2 (34), 173–180.

Wilkaniec Z., Szymaś B. and Wyrwa F. (1996) Permanent meadows as a food and habitat basis for bees. *Roczniki Akademii Rolniczej* w Poznaniu 284, Rolnictwo 47, 105–110.

Wyłupek T. (2002) Floristic diversity and natural value of grassland in Por river valley. *Fragmenta Agronomica*, 19, 1 (73), 213–223.

Zarzycki J. and Misztal A. (2010) Abandonment of farming practices: impact on vegetation. *Grassland Science in Europe* 15, 133–135.

Żurek H. (2001) The influence of various modes of rotation grazing on the productivity and plant cover of a dairy cows pasture on peat-muck soil. *Water-Environment-Rural Areas* 1, 2 (2), 27–46.

# Past and future of European grasslands. The challenge of the CAP towards 2020

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# Abstract

Grasslands are important in the European Union (EU) agricultural area (39% in 2007) although the area of permanent grasslands has decreased probably by about 15% or more in the last 50 years. This decrease of the permanent grassland area has several negative environmental consequences including biodiversity loss, Green House Gas (GHG) emissions and soil erosion. The importance of EU dairy and meat product exports on the world market is declining. The progressive liberalization of the Common Agricultural Policy (CAP) is certainly one of the factors explaining this decrease. In contrast, feed imports have considerably increased in the last 50 years (+400%) and are now equivalent to about 10 to 25% of the total EU-27 permanent grassland production in ME and CP respectively. The CAP has evolved a lot since the beginning in 1962. Two major trends are progressive liberalization and higher concern for the environment. The present proposals of the European Commission (EC) continue some of the present objectives and also include new ones that take into account the enlargement to new Member States. The mechanisms designed by the EC for reaching these objectives, especially for the 'Greening component' of pillar 1, are though very unlikely to succeed. The definition of permanent grassland is far too restrictive and inadequate. Semi-natural grasslands and High Nature Value farmland are not specifically targeted although they are the most valuable ecosystems of the farmland area. Greening measures are too general, not targeted, not contractual and based on one-year farmers' commitments. They do not include farmers' advice and training, and a convincing control system. Most CAP expenditures should shift from pillar 1 to pillar 2 and the agri-environmental scheme. In the medium term, CAP expenditures should support the emergence of a market of public goods (biodiversity, landscape and carbon storage mainly) supported by pillar 2 budget (public money for public goods). Other challenges like protein self-sufficiency for animal feeding, food waste reduction and improvement of the fatty acid composition of animal products and its consequences for human health should be taken into account in CAP reform proposals. An increase of the grassland area and the implementation of an efficient system for biodiversity conservation in agriculture are highly desirable.

Keywords: policy evolution, global market, greening, agri-environment, public goods

# Introduction

The world is changing rapidly. The European Union has grown with the successive enlargements, the characteristics and priorities of the EU-27 farms and agricultural regions are now very heterogeneous, globalization has profound effects on all economies of the world and European agriculture is not by-passed, world market prices of agricultural commodities are increasing while fluctuating a lot, the future of the European currency is uncertain, public expenses and debts have reached alarming levels in several EU countries, the global human population is growing very fast inducing high demand for food (Delgado, 2005; Kearney, 2010), agro-fuel production is increasing, the purchasing power of emerging economy countries is growing rapidly creating new markets including for luxury and high-quality products. Main global challenges are related with the financial, economic, demographic, food, energy and ecological crisis. Among ecological problems, the loss of biodiversity is extremely fast, including in Europe, and especially on European farmland. Atmospheric changes (climate and  $CO_2$  content of the atmosphere) are fast too, which have positive and negative influences on agricultural production and biodiversity. The CAP has not the objective to contribute to solving all these problems but it is influenced by them. It can contribute to solve a part of these problems and its reform has to be thought in this context. The CAP is not only an instrument for food security and for income support of farmers anymore, it has progressively become a tool for answering wider challenges. There is thus a need for a major reform of the CAP.

#### Importance and evolution of grassland-based systems

Permanent grasslands (PG) cover over 57 million ha in the EU-27 (2007), temporary grasslands (TG) about 10 million ha (Eurostat). Together, they occupy about 15% of the whole EU territory and about 39% of the European Utilized Agricultural Area (UAA). They are managed by about 5.4 million holders, i.e. about 40% of all European farm managers. Among these farms managing permanent grasslands, many are still small. About 41% have an Economic Size Unit (ESU) lower than one (very small farms). There were about 134 million Livestock Units (LU) of total livestock and 78 million LU of grazing livestock (59%) in the EU-27 in 2007. The vast majority of them are located in the EU-15. In the grazing livestock population (in LU) in the EU-27, 82% are cattle and 14% small ruminants (sheep and goats). Dairy cows account for 31% and other cows (mainly suckler cows) for 16% in the total LU of grazing livestock. Two thirds of cows are thus dairy cows, one-third other cows. Beef and yeal, sheep and goat meats amount to 11% and milk to 14% of the total agricultural production value. Farms specialised in grazing livestock employed about 21% of total EU-27 agricultural labour force in 2007 (Eurostat, 2010 in Osterburg et al., 2010). In the EU-6 (Benelux, France, Germany (GFR), Italy), losses of the PG area are estimated at about 30% and 7 million ha between 1967 and 2007 (Eurostat). In the EU-15, losses are probably closer to 15% or 10.5 million ha in 50 years (FAOSTAT). In the same period (1961–2009), the maize area more than doubled, it gained 1.2 million ha in France, Germany and Benelux while the total cereal area remained almost stable (FAOSTAT). The dairy cow population fell by 10 million head in the EU-9 between 1975 and 2007 (decrease of 40% of the population of 1975). This decline started after the implementation of the milk quotas in 1984. In contrast, the suckler cow and sheep populations increased, by about 3 and 8 millions head respectively, during the same period in the EU-9. In the former communist countries, cattle and sheep numbers declined sharply, by at least 50%, in the 1990s and started to stabilize or to increase slowly in the first years of the 21<sup>st</sup> century. The total number of agricultural holdings in the EU-9 was reduced by almost 50% in 30 years (1975–2007). The decline of dairying specialists was very important (72%) while numbers of cattle rearing and fattening specialists and sheep, goats and other grazing livestock specialists were much more stable (3% decline and 15% increase respectively). The average size of grazing livestock holdings almost doubled during that period (Peeters, 2010).

The structure of production has thus evolved a lot in the last 50 years, and farming practices evolved even more. Nitrogen fertilization was almost unknown in grassland at the beginning of the period. In North-West Europe, annual organic and inorganic fertilizations reach now regularly 300 kg N ha<sup>-1</sup> or more in highly intensive dairy farms. Stocking rates (LU ha<sup>-1</sup>) increased a lot per ha of UAA and per ha of PG. That was possible because of the increasing use of fertilizers and concentrate feeding. Techniques of forage conservation improved a lot,

which induced a shift between hay- and silage-making. Grasslands were thus cut earlier and more often for producing higher quality forage. Drainage made possible the intensification of wet and valley bottom grasslands. Re-sowing and over-sowing with improved cultivars of grasses and legumes improved the productivity of the sward. Weed control with herbicides eliminated most dicotyledon species in intensive grasslands, making more room for productive and high-quality grasses. Irrigation of temporary grasslands became common in the South of Europe and other summer drought areas. A significant proportion of PG was converted into forage maize and cereal crops. Another part was lost because of urbanization or afforestation. Very large areas were afforested from the 1990s for instance in Spain and Portugal with CAP supports. Farming systems specialized and this induced a divorce between livestock rearing and cropping. Breeding produced specialized cattle breeds, some for dairy, others for meat production (Peeters, 2009). The conclusions of the 'Dillon Round' of the General Agreement on Tariff and Trade (GATT) negotiations in 1962–1963 included the acceptation by European negotiators of free-tax imports of protein-rich feedstuff for animal feeding. That induced a fast increase of industrial monogastric (pig and poultry) production and blocked any further development of legumes and protein crops in Europe.

All these technique improvements and farm structure modifications increased yields, production and quality but had also negative consequences on the environment: massive loss of biodiversity, nitrate and phosphorus water pollution, high fossil energy use and GHG emissions.

### Some environmental consequences of the evolutions

The conversion of permanent grasslands into arable land has several negative environmental consequences, i.e. reduced soil C storage, water pollution, soil erosion and decline of biodiversity. Some examples are given below (Dillon, 2010). For European soils, a modelling approach showed that conversion of arable land to grassland leads to an estimated increase of Soil Organic Content (SOC) content in the order of 1.44 tonne C ha<sup>-1</sup> yr<sup>-1</sup>, whereas existing grasslands still build up SOC content at a rate of 0.52 tonne C ha<sup>-1</sup> yr<sup>-1</sup> and arable lands lose SOC at a rate of -0.84 tonne C ha<sup>-1</sup> yr<sup>-1</sup> (Vleeshouwers and Verhagen, 2002). Calculations by Janssens et al. (2005) showed comparative results with a build-up of SOC under grasslands of 0.6 t ha<sup>-1</sup> yr<sup>-1</sup> and a loss of 0.7 t ha<sup>-1</sup> yr<sup>-1</sup> for arable soils, on average and for a European context. Grasslands positively mitigate soil erosion and runoff through a permanent cover of the soil and a dense rooting system. Estimated average erosion rates, in a European context, are much lower for grasslands (0.3 t ha<sup>-1</sup> yr<sup>-1</sup>) compared to arable lands (3.6 t ha<sup>-1</sup> yr<sup>-1</sup>) (Cerdan et al., 2010). Grasslands have a better effect on water quality and water resources than other crops (Briggs and Courtney, 1989; Benoit et al., 1995; Benoit and Simon, 2004). Permanent grasslands can protect water tables in areas with a high risk of nitrate leaching (Bossuet et al., 2006). They can store organic N in their large organic matter soil pool (Brogan, 1966; McGrath and Zhang, 2003; O'Connell et al., 2003), lowering the risk of N loss to water. The increasing land use intensity has caused a severe decline in biodiversity related to the agricultural landscape (Donald et al., 2006; Plantureux et al., 2005; Peeters, 2009), replacing many specialist species by a handful of thriving generalist species (Walker et al., 2004). Semi-natural grassland areas often show important natural values (Dauber et al., 2006). Due to the threats associated with both intensification and abandonment, they are though among the most vulnerable ecosystems (Veen et al., 2001). Although intensively managed grasslands support less biodiversity, they are often considered to be more positive for biodiversity than other agricultural land uses, e.g. arable crops (Peeters, 2009; Plantureux et al., 2005). In permanent grass-dominated landscapes, some arable cropping may though increase biodiversity.

## Evolution of imports and exports on the global market

In a short-term study (1999–2008), von Witzke and Noleppa (2010) showed that total EU agricultural exports have slightly decreased (in volume) while imports have grown considerably (+24%) during the period they considered. They estimated that the EU imported the equivalent of 35 million ha of 'virtual land' (land necessary for producing a given tonnage of commodity on the basis of regional yields) in 2007–2008, almost 40% above the value for the years 1999–2000. Bovine meat exports went down by 84% (from 0.56 to 0.09 million tonnes) while other meat exports (mainly pig meat) have risen by about 10%. In 2008, imports of bovine meat were about 2.7 times bigger than exports. It was almost the opposite in 1999. The EU is still a net exporter of total meat (bovine + other meats) but annual exports decreased from 2.2 to 1.4 million tonnes. Dairy product exports have declined by about 10% and imports by 14%. The EU was still a net exporter of about 2.09 million tonnes of dairy products in 2008. At the same time, feeding stuff imports from soybeans increased by 45%. Soybean represents the largest 'virtual land' import in the EU with 19 million ha or 39% of the total virtual land imports in 2008.

Over a longer period, between 1961 and 2008, feed imports have increased in the 27 countries of the present EU by about 400% (in tonnes) (FAOSTAT). The main increase occurred between 1961 and 1992 (500% of 1961 tonnage), after which a slightly downward trend of total import was observed. Soy became the main product of feed imports (83% in 2008), showing a continuous linear increase. Imports of other commodities decreased. Brazil, Argentina and USA are the main export countries for the EU-27. Expressed in N and P imported with feed products, the evolution of imports is even more important than in tonnes of products. N imports increased by 600% and P imports by 450%. Expressed in metabolizable energy (ME) and crude protein (CP), annual imports amounted up to 490,973 TerraJoules ME and 17 million t CP in 2008. These amounts of imports have been converted to equivalent of the total EU-27 grassland area on the basis of two annual grassland yield hypotheses (5 or 10 t DM ha<sup>-1</sup>), and other assumptions related to the proportion and nutritive values of grazed, harvested for hay, harvested for silage forages. Feed imports are equivalent to between 7 to 14% of the total EU-27 grassland area on a ME basis, and between 17 to 35% on a CP basis (Swolfs 2011 and own calculations).

## Main drivers of evolutions

The progressive liberalization of the CAP and concessions at the World Trade Organization (WTO) negotiation have reduced export subsidies and import duties on many products. Domestic price supports have also been reduced. The high conversion rate of the euro against the dollar made exports more difficult and imports cheaper. The 'Every Thing But Arms' agreement (2001) with the least developed countries contributed also partly to the growth of net imports. All these factors induced a decrease of exports and an increase of imports (von Witzke and Noleppa, 2010).

Regarding beef meat, production was reduced because of the implementation of the milk quotas and the continuous increase of dairy performance per cow that led to a strong decrease of dairy cow numbers. The decrease of meat production associated with dairy systems has not been totally compensated by the increase in production from beef breeds.

Changes in cultural feeding habits of European consumers had also an effect. EU citizens eat more 'white meat', less 'red meat' and more processed or prepared food 'away form home' (Duquesne *et al.*, 2006; European Commission, 2003). Pig meat accounts for about 44 kg, beef and veal 20 kg and poultry 23 kg per head and per year (European Commission, 2003). White meat is cheaper than red meat notably because pigs and poultry are largely

fed with cheap imported feedstuff that are for instance by-products of oil extraction and of other agro-food industries, and are imported without or with a low level of duties notably from developing countries where land and manpower are cheap. The evolution of the live-stock population of the 27 European countries between 1961 and 2008 shows a significant increase in the number of pigs and chicken (about +60%) but reductions in the number of cattle and sheep (about -11%). The volume of pig and poultry meat increased even more (+138 and 393% respectively) because of productivity gains.

#### Periods and trends of the evolution of the CAP

Several periods can be recognized in the evolution of the CAP.

**Intensification and modernisation of farming in a planned economy.** The objectives of the CAP, defined in the Treaty of Rome, were increasing agricultural productivity, ensuring a fair standard of living for the agricultural community, stable markets and an affordable supply of food at a reasonable price for European consumers. The 'Community preference' was also engraved in the Treaty. Between 1960 and 1980, the priorities were mainly increasing yields, production, and the self-sufficiency of the EU. At that time, target and intervention prices of agricultural commodities were fixed each year by the Council of Ministers of Agriculture and supported by the CAP budget. This period ended with the emergence of almost permanent surpluses of the major farm commodities, which was, in a way, the expression of the success of this policy!

**Restructuring and increasing farm size.** Between 1970 and 2011, the necessity of restructuring and increasing farm size was translated into several programmes. In 1968, the 'Mansholt Plan' aimed at removing small farmers from the land and at consolidating farming into a larger, more efficient industry. This Plan was never adopted as such but, in the next decades, compromise solutions encouraged and stimulated modernisation of agricultural holdings, cessation of farming activities and training of farmers.

Development of the Rural Development Policy (RDP). An outline of a RDP was adopted in 1975 with the implementation of the Less Favoured Area (LFA) programme. This programme aimed at maintaining farming in mountain and other marginal areas where production conditions are difficult. Grassland farming of these areas benefited a lot from this programme. Another major step was the launching in 1992 of the Agri-Environmental Measures (AEM) for all Member States (MS) (AEM were first introduced as optional measures for MS in 1986). The RDP gained coherence and visibility after 1999. The 'Agenda 2000' reforms encouraged many rural initiatives while also helping farmers to diversify, to improve their product marketing and to restructure their businesses if needed. The diversification of activities in rural areas such as the development and marketing of high quality products, rural tourism, conservation of the environment or cultural heritage, supported by the RDP, were opportunities to supplement agricultural income and open up new prospects for rural life. The importance of the RDP increased progressively over time. The Greening of the 1st pillar of the CAP, in the present proposals of the EC, can be also considered as an expression of this trend, even if the propositions for the Greening of the CAP are not formally included in the RDP.

**Controlling and decreasing expenditure.** In the early 1980s, the EU had to deal with surpluses of many products. Some of which were exported with the help of subsidies; a large part of these surpluses had to be stored within the EU. These measures were expensive, distorted world markets and became unpopular with society. In order to reduce surpluses, production limits were implemented in some sectors, notably for dairy production by the adoption of milk quotas in 1984 and of sugar quotas in 1968. The policy of reduction of

the total budget of the CAP is still on-going. The proportion of the CAP budget in the EU budget decreased progressively being 90% in 1970, 60% in 1988, 52% in 1992, 45% in 1999 and 47% in 2006. In the 'Agenda 2000' reform of 1999, the budget was capped to control expenditure.

Liberalization. In 1992, the 'MacSharry Reform' involved a dramatic reduction of agricultural prices to get closer to global market prices. Price support payments were cut for beef, cereals and milk; cuts for milk were delayed until 2006. It was intended to compensate income loss for farmers by paying direct aids per hectare or an animal head basis. The reform created 'set-aside' payments to withdraw land from production, payments to limit stocking rate levels, and introduced measures to encourage retirement and afforestation. These measures intended to limit the rise in production. This reform permitted more freedom and more space for initiatives for agricultural entrepreneurs. The reform of 1992 was a shift from a system of price supports to a system of income supports. In 1999, the 'Agenda 2000' reforms stimulated even more farmers to be more reliant on the market. In 2003, the 'Fischler Reform' deepened the 1992 and 1999 reforms by reinforcing the market-oriented character of the CAP and the importance of environmental protection. Four new principles were added into the previous systems: decoupling, cross-compliance on the 1st pillar, modulation and partial re-nationalisation. Aids were decoupled from specific production and this reduced market distortions. The reform encouraged more initiatives by farmers. The phasing-out of the sugar (2005) and milk (2015) quotas is another expression of this growing liberalization trend, of a transition from a planned economy towards more free market.

Environmental protection. In the reform of 1992, a 'cross-compliance principle' was introduced; aids were only eligible if farmers respected basic rules including for the respect of the environment. Several rural development measures were developed, notably to encourage environmentally sound farming through the agri-environmental scheme. AEM were defined as practices that go beyond Good Agricultural and Environmental Practices (GAEP). In 1999, the 'Agenda 2000' reforms improved incentives to farm in an environmentally sensitive way. In 2003, the 'Fischler Reform' consolidated also the environmental aspects initiated by the 1992 and 1999 reforms. The cross-compliance principle was significantly reinforced, including a mechanism intended to maintain the permanent grassland area. Decoupling reduced perverse effects of policies on the environment, notably the encouragement to convert permanent grasslands into forage maize crops. Modulation permitted a transfer of pillar 1 budget to the RDP budget, including for AEM. In 2008, the 'Health Check' of the CAP confirmed this trend and defined four 'new challenges' that should attract more funding: climate change, renewable energy, water management and biodiversity. From the 1990s until today, pollution control and biodiversity restoration were constant and increasingly important concerns. An environmental regulation, the Nitrate Directive, was adopted as a powerful means for limiting water pollution. The decline of biodiversity in agriculture could though not be stopped. Since that period, the reforms for better environmental protection and more liberalization were developed in parallel.

## The 'Sapir report'

The Sapir report (Sapir *et al.*, 2003) was ordered in 2002 by Romano Prodi, the President of the European Commission, for contributing to the definition of an economic strategy for the EU in the first decade of the 21<sup>st</sup> century. It does not focus on the CAP but is an agenda for a growing Europe. It recommends that more expenditure would be channelled to growth-enhancing activities such as research and development (R&D) and education. As a significant proportion of the budget of the EU is currently spent on agriculture, and there is little

prospect of the budget being increased, this would necessitate reducing CAP expenditure. The report considers also that the CAP does not seem consistent with the Lisbon goals, because its value-for-money contribution to EU growth and convergence is lower than most other policies. The report states that reducing income disparities among MS is a matter of absolute urgency and priority in the enlarged EU.

### Present propositions for the CAP reform: good objectives, disappointing methods

On 12 October 2011, the EC presented a proposition for a reform of the CAP after 2013 (European Commission, 2011a; 2011b). This document identifies challenges related with 'the agriculture's productive capacity, the increasing diversity of agriculture and rural areas following successive enlargements, and the demands by EU citizens on the environment, food safety and quality, healthy nutrition, animal health and welfare, plant health, the preservation of the countryside, biodiversity and climate change. Three broad objectives of the future CAP are defined: 'Viable food production', 'Sustainable management of natural resources' and 'Balanced territorial development'. Most changes are related to pillar 1. A new 'Basic Payment Scheme' will introduce more equity in the payments per ha within and between MS. A compulsory 'Greening' component will support farmers for respecting agricultural practices beneficial for the climate and the environment. MS may grant an additional payment from pillar 1 for areas with natural constraints. This support has to be limited to 5% of the national envelope. Part of the budget will target young and small farmers. MS would have the possibility to spend limited amounts of their envelope (maximum 5%) on 'coupled' payments linked to a specific product. Limited transfer would be allowed between pillars 1 and 2 or pillars 2 and 1. The cross-compliance principle would be reinforced. Capping would limit payments that very large farms can receive.

The general objectives of the 2011 CAP reform proposals of the EC correspond to longterm trends of the evolution of the CAP, to demands of society and they probably can be supported by a large part of the scientific community (Peeters, 2008). New ideas have been introduced compared to the former CAP. More fairness in the distribution of supports is certainly something that could increase cohesion of the EU, support the income of small farmers of the new MS and of extensive farmers of all MS and could be used for enhancing the environment, protecting biodiversity and landscapes. A successful transition of the economies of the new MS is vital for all EU countries. CAP mechanisms should target farmers of these countries for helping them to develop a modern and sustainable agriculture while protecting the environment. Capping is also a tool that can contribute to distributing supports to farmers who need them the most. If a consensus can be found on the objectives, mechanisms for reaching these objectives can be discussed!

The structure of the CAP budget must be totally reorganised. Direct payments, even if better distributed among and within MS, cannot be socially justified anymore in a context of public expense reduction and economic difficulties. Farmers should not be paid just for farming! They should earn income from their farming activities that should be as much as possible economically viable. This viability is though not always guaranteed. It can be increased by complementary activities that must also be based on the reality of a market. Farmers should notably be rewarded for the positive actions they undertake for a sustainable management of natural resources and for the delivery of ecosystem services. A market should be initiated and organized by the CAP for the production of public goods and services. This market should be largely financed by public money (Public money for public goods!). This implies a change of paradigm of the CAP. The CAP must be simplified and its efficiency increased. The recent proposals do not really go in this direction. The greening component of the 1<sup>st</sup> pillar introduces confusion between the objectives of the 1<sup>st</sup> and the 2<sup>nd</sup> pillars. If the greening component of pillar 1 is considered as a 'light' version of AEM, AEM and LFA programmes would have both 1<sup>st</sup> and  $2^{nd}$  pillar components. The articulation between the green component of pillar 1 and the environmental scheme of the RDP is not defined. Even more importantly, the CAP must demonstrate good value for money to tax payers. That implies the delivery of public goods and the implementation of an effective system for this delivery. The budget devoted to the greening is important (about 30% of the national envelopes of direct payments) and could triple the amount spent on agri-environment compared to the present situation if the budget of AEM is maintained. The 3 measures of the greening component - maintaining permanent pasture, crop diversification and maintaining an 'ecological focus area' of at least 7% of farmland – are welcome in their principle. Supporting permanent pastures is highly justified for many reasons mentioned above. The environmental benefits of this measure will though be limited. The definition of permanent grassland by European Commission (2011a) – 'land used to grow grasses or other herbaceous forage naturally (self-seeded) or through cultivation (sown) and that has not been included in the crop rotation of the holding for five years or longer' - does not take into account the vast grazed areas that include high proportions of trees and/or shrubs and that have been used for centuries in different areas of Europe from Sweden to the South of Spain and to Greece. Grazed woodlands, Calluna heather and other Ericaceae communities in the lowlands and in mountains, Mediterranean matorral, the Spanish Dehesa and the Portuguese Montado for instance would be excluded from supports and they are among the most precious and biologically rich, grazed ecosystems of Europe. They are also storing carbon in higher amounts than other grazed ecosystems. On the other hand, large areas of grasslands are regularly resown without taking part in crop rotations. The soil cover is always grass but the vegetation is not permanent grassland. These grasslands provide much lower environmental benefits and are species-poor. The definition should only include grasslands that are not regularly ploughed or chemically destroyed and reseeded. The greening measure for grassland and rangeland conservation should target specifically semi-natural vegetation i.e. managed ecosystems dominated by indigenous or naturally occurring grasses, other herbaceous species and/ or shrubs which are grazed or have the potential to be grazed (Allen et al., 2011). These ecosystems are not substantially modified by fertilisation, liming, drainage, herbicide use, introduction of exotic species and over-sowing. Forestland that produces, at least periodically, understorey vegetation that is grazed should also be included. Compared to semi-natural grasslands, lower level of subsidies should support more intensively used permanent grasslands. Permanent grasslands can be defined as: Land, and the vegetation growing on it, devoted to the production of introduced or indigenous forage for harvest by grazing, cutting, or both. Forage is composed of perennial or self-seeding annual plants kept indefinitely. It may include either naturalized or cultivated forages. At times woody species may be present (Allen et al., 2011). The definition of intensively used PG implies more frequent defoliations and higher stocking rates and productions than semi-natural grasslands. Simple maintenance rules should be defined in the support system and controlled by a credible monitoring and evaluation procedure. Legume-based temporary grasslands could be supported too, at a lower rate than intensively used PG.

The environmental objectives and management of the 'ecological focus area' are not defined. Terms like 'field margins, hedges, trees, fallow land, buffer strips, afforested area' are rather vague and the biodiversity of these areas can be of high-value, but could also be of low-value, sometimes with harmful elements. These areas should be carefully defined, in-field and on field edges, include all types of ecological infrastructures that are beneficial to biodiversity and their management should be checked and the results evaluated. That implies higher levels of control than with the present  $1^{st}$  pillar measures and an implementation philosophy closer to the  $2^{nd}$  pillar.

The greening component should support as a priority those farming systems that provide environmental public goods and services. **High Nature Value** (HNV) farming systems are one of them. Their persistence is threatened by a low profitability. HNV farmland is often managed by small farmers that the reform would like to support. However, the proposition of the EC insufficiently includes HNV. If it is recognised amongst the main objectives of the RDP, there is no mention of any tool specifically focused on it. This tool could possibly be integrated in a 1<sup>st</sup> pillar component (Beaufoy and Marsden, 2011)

MS may grant an additional 1<sup>st</sup> pillar payment for **areas with natural constraints**, for a very limited amount of their national envelope, but no clear environmental objectives are associated to this measure. These payments should be merged with those of the LFA scheme of the RDP and used for financing HNV farming on a simple, clear and effective basis. That would result in giving a stronger content and a clearer environmental objective to the LFA programme (The present definition: 'a broad-scale mechanism for maintaining the countryside in marginal areas' is very general and has limited environmental relevance).

Farmers adopting appropriate management practices in **Natura 2000** areas should receive additional supports in the green part of pillar 1. These supports should be complementary to pillar 2 existing payments for Natura 2000. Such actions would create the conditions for safeguarding these high biological value habitats. Many HNV farmlands are in Natura 2000 areas. The combination of supports of both programmes should be managed in an adequate way.

The **cross-compliance principle** and the **Good Agricultural and Environmental Conditions** (**GAEC**) definition must be a strong basis on which other schemes can build. They must be based on the polluter pays principle. This includes the Birds and Habitats Directives, the Water Framework Directive and all new environmental directives on soils and pesticides for instance that can be designed in the future. Because of their very nature, they **must be respected by all producers**. Adequate penalties (in the form of taxes (charges on over-fertilization, species or habitat destruction and habitat degradation) or other financial penalties (proportional reduction in compensatory payments or investment aid)) should be applied if not respected. Below a certain level of GAEC, farmers should not be allowed to operate and should cease any practices that are harmful to the environment.

**All RDP programmes should have clear objectives including environmental objectives** like AEM, LFA, investment in physical and human capital and the Leader axis. Special attention should be paid to the avoidance of distortion effects of these policies, especially investment supports and LEADER projects, on the environment.

The current **agri-environmental scheme** has had a positive effect on the environment by slowing down the degradations, by maintaining a situation or by restoring biodiversity and landscapes. Its effect was though insufficient, as recognized in the EU Biodiversity Strategy. There are several reasons for this, including issues with national or regional scheme design, the targeting of the measures, the way they are implemented, a lack of farmer's advice, low administrative capacity, low payment levels and insufficient budget. The budget for AEM should thus not be decreased, it should increase, but the proposals of the EC do not guarantee this increase. The higher budget for RDP would be undermined if funds were transferred from pillar 2 to pillar 1. This should not be permitted. Improving the efficiency of AEM

is also necessary. Result-oriented AEM instead of mean obligation measures are likely to be more efficient when they are applicable (Matzdorf *et al.*, 2008; Oppermann, 2003; de Sainte-Marie, 2009; Wittig *et al.*, 2006). In each country, AEM should be better targeted. 'From an environmental perspective, the more tailored the measures are to specific environmental needs and the more they are targeted at the locations in which action is needed, the more effective and efficient they are likely to be in achieving their objectives' (Hart and Baldock, 2011). Increasing farmer's advice and training on AEM and increasing monitoring and evaluation will require greater administrative efforts and a somewhat larger part of the budget, but these conditions are necessary for ensuring effectiveness, efficiency and good value for public money (Hart and Baldock, 2011).

The present proposals of greening measures will not deliver important environmental benefits because they are too general and not targeted, they will apparently not include training, monitoring and evaluation of the results. It has been shown that only targeted measures can be efficient for biodiversity restoration and conservation (for instance: Bretagnolle *et al.*, 2011). General and broad measures, like those corresponding to the management rules of pillar 1, are not. Non-contractual agri-environmental actions will thus most likely not deliver significant results! Most measures require long-term adoption for reaching consistent results. The one-year basis of the EC proposal does not fit with this criterion. Multi-annual commitments should be considered.

If all these aspects are not taken into account in the final version of the reform, the credibility of the 'greening' component of the CAP and of the whole CAP will be threatened. Independently of the reform proposals and the previous comments, a change of paradigm is needed for the CAP. The CAP budget should move from income support to a public good market financing. That will give a new legitimacy to this policy. The largest part of the CAP budget should be transferred to pillar 2, with a lowering of co-funding rates in less developed regions (which is included in the EC proposals). The remaining part of pillar 1 budget (for instance about 20% of the total CAP budget) should be mainly kept for stabilizing income in case of high price volatility (safety net). Pillar 2 budget would become the basis for creating a true market for public goods and services and in priority for biodiversity and landscape conservation, carbon storage and water quality. On the supply side, initiatives could come from farmers advised by experts (research organisations, specialized NGO, R&D offices) for proposing (offering) public goods and services. On the demand side, the 2<sup>nd</sup> pillar of the CAP would be the main source of payments but other public authorities (national, regional, local), private companies, individuals or group of individuals could evaluate these proposals and decide to pay (to buy) them or not. Improved AEM should remain the reference and a source of inspiration for this market. This system based on private initiative, creativity and efficiency would boost the protection of the environment and stimulate rural life. It would create a vibrant countryside, create new jobs and increase contacts between the agricultural sector and other stakeholder types.

Despite large heterogeneities of the EU agricultural sector, I consider that the CAP should remain one of the main policies of the Union. That implies a large degree of 'subsidiarity' for adapting the principles and regulations to the diversity of production conditions. That will also necessitate a simple and easily understandable structure of the CAP. A single European agricultural policy is not only necessary for avoiding competition distortions between regions and countries but also because the diversity of approaches between MS has led to creative common programmes that drew up the whole community of countries instead of leading them to adopt the lowest common denominator. This positive dynamic deserves to be maintained.

### Other challenges for the future

**Food security** is never guaranteed forever, it must always remain a concern because of its strategic importance. In order to meet its own demand for food, feed, fibre and agro-fuel, the EU uses a virtual land area in overseas countries of about 35 million hectares, equivalent to about twice the size of the UAA of Germany (von Witzke and Noleppa, 2010). This expansion in demand for overseas land by the EU is leading to deforestation, biodiversity losses (e.g. in the rainforests of South America and Indonesia, in species-rich grasslands of the Pampa, the Campos and the Cerrado in South America) and GHG emissions. The main shortcoming of the EU with regard to food security is the **protein dependence** for animal feeding. These imported feeds are largely used for pig and poultry feeding but also for the supplementation of maize and other cereal-based protein-poor rations for dairy cow feeding and beef cattle fattening. Protein independence can only be achieved by a combination of several means: decrease of meat consumption (in the EU; total percapita protein consumption is about 70% higher than recommended and average intake of saturated fatty acids is about 40% higher), food waste reductions, partial shift from pork and poultry meat to sheep and beef meat, supports for larger use of grasslands, developing crops of annual and perennial legumes, substitution of maize and soybean by grass feeding for ruminants. Reduction of food wastes can also have an impact on the EU land structure. Westhoek et al. (2011) identified three strategies for the livestock sector: (i) increasing resource efficiency, (ii) consuming less or different animal products, and (iii) producing with fewer local impacts. Present policies are encouraging the first strategy but regarding the second strategy, policies are practically non-existent, and with respect to the third strategy, policies are usually secondary to free market policies. The CAP could be an efficient tool for orientating the livestock sector in the right direction.

Regarding global food security, it is questionable if the EU will remain a major actor of **milk and meat exports on the global market** in the future. Brazil has an important potential for dairy production. Countries of the Mercosur have already an important capacity for beef meat exports and are developing their monogastric meat production capacity. Ukraine and Russia have a significant potential for (dairy and) beef production and exports. China is developing quickly its dairy production (FAOSTAT; Godfray *et al.*, 2010; Thornton, 2010). The future role of the EU is more likely to be to export high-quality products like PDO cheeses and other processed food. The EU will thus not significantly contribute to feed the world and further intensification of EU agriculture should not be a goal. The EU has to better use its own resources, including grasslands that are a cheap source of protein and energy for producing animal products.

**Human health** remains a concern in Europe despite the increase in life expectancy. Cardiovascular diseases, inflammatory and auto-immune diseases (allergies), and obesity can be induced by unfavourable characteristics of fatty acids in animal products. Compared with grain-fed beef or milk, grass-fed beef or milk are lower in total fat, lower in saturated fatty acids (Couvreur *et al.*, 2006) linked with coronary heart diseases (CHD), higher in total omega-3 (and has a healthier ratio of omega-6 to omega-3 fatty acids (1.7 *versus* 5-14)), higher in conjugated linolenic acid (CLA) (cis-9 trans-11) (Dhiman *et al.*, 1999) that is anticancer, and higher in vaccenic acid (which can be transformed into CLA) (Duckett *et al.*, 2009). In human organisms, omega-3 fatty acids protect against vascular diseases, induce elasticity of blood vessels and blood fluidity, are essential for the development of retina, brain and the nervous system, are necessary for a normal growth and are associated with other physiological functions in all tissues. Omega-6 fatty acids in excess can prevent omega-3 from playing their role in cardio-vascular protection and provoke pain and inflammatory diseases like asthma and arthritis. Excessive consumption of omega-6 fatty acids induces increased development of fat tissue from childhood, obesity, an increase of inflammatory and auto-immune diseases (allergies), dementia and some cancers (Simopoulos, 2002). Grass is rich in omega-3 and poor in omega-6 (grazed grass omega-6/omega-3 ratio = 0.4; grass or legume hay and silage ratio = 0.7) while cereals and maize silage (ratio = 14) and soybean meal (ratio = 5) have very different characteristics (Simopolous and Robinson 1999). Grass-fed beef meat is about 4 times lower in total fat than grain-fed meat (Rule *et al.*, 2002). It is also lower in saturated and omega-6 fatty acids. Omega-3 fatty acid content of meat decreases during the fattening period if grains are abundant in the animal diet. It is reduced by about 50% in 2 months and by about 75% in 3 months (Duckett *et al.*, 1993).

Obesity is induced by the consumption of energy dense food (cereals, bread, pasta, rice), sugar (bakery products, sweets, soda), saturated fatty acid (grain-based animal fat), a high omega-6/omega-3 ratio and reduced physical activity levels (World Health Organization, 2012; Simopoulos, 2002). Obesity can itself induce diet-related chronic diseases, including type 2 diabetes, cardiovascular diseases, hypertension, stroke and certain forms of cancers. Obesity occurrence is increasing in developed countries, with 31% of the population in the USA, 23% in the UK, 12% in Belgium (but only 3% in Japan) being considered to be obese. By 2020, it is estimated that about 75% of the population will be overweight or obese in the USA (OECD, 2011).

Each year, about 90 million tonnes or 179 kg per capita (in 2006) of food wastes are generated in the EU-27 (European Commission 2010). These values are expected to rise. Households produce the largest fraction of food waste (about 42% of the total) about 38 Mt and an average of about 76 kg per capita and per year; the manufacturing sector almost 35 Mt per year and 70 kg per capita; the wholesale/retail sector around 4.4 Mt close to 8 kg per capita with an important discrepancy between MS; the food service sector 12.3 Mt or an average of 25 kg per capita. In the food service sector, there is a notable difference between the EU-15 at 28 kg per capita and per year (due to a higher trend of food waste in the restaurant and catering sector) and the EU-12 at 12 kg per capita. Food discarded in UK households represents 25% of food purchased (by weight) (WRAP 2009 and 2010). According to WRAP (2009), in UK households, about two thirds of wastes (approximately 565 euros per household) are avoidable. It is estimated that 30 to 50% of the total food produced is wasted (Parfitt et al., 2010; WRAP, 2009; 2010). These wastes have important consequences in terms of social justice, finance of citizens, economic activity, energy consumption, GHG emissions, biodiversity conservation and land use. Policy options for reducing wastes are described for instance in European Commission (2010b). In addition to these ideas, two ways should be explored: higher development of bio-methanization of a part of these biowastes, recycling of a part of wastes in animal feeding. Recycling could contribute to reduce the feed protein dependence of the EU. This last solution is part of an agricultural policy. Among other challenges for future farming systems, energy consumption and production, water quality, reduction of GHG and NH<sub>2</sub> emissions, and urbanization control are important. A 'Resource efficient Europe' preserving its existing biodiversity implies a significant transition in agriculture and alignment of the CAP to a low carbon economy.

All these challenges should be taken into account in agricultural policies and can have an impact on the PG area.

## Conclusions

In every policy document, **the support to 'permanent grasslands and rangelands' should include ecosystems dominated by shrubs and/or trees** and that are traditionally grazed. Given the fast erosion of biodiversity in the EU, specific measures should support the maintenance and the restoration of semi-natural grasslands, within and outside Natura 2000 areas, and the farming systems that ensure their persistence, the HNV farming systems. More intensively used permanent grasslands and, to a lesser extent, legume-based temporary grasslands should also be supported because they protect natural resources and provide ecosystems services compared with arable land, although to a lesser extent than semi-natural grasslands.

Only AEM and greening components that target a species, a group of species, or a habitat can be efficient. AEM and greening components can also be designed to support a broad farming system, e.g. extensive sheep grazing system or other HNV farming systems, that can deliver real environmental benefits. With the exception of the support of these particular farming systems, general horizontal and not targeted measures are not efficient. Farmers' advice and training are essential because they do not yet sufficiently consider themselves as providers of biodiversity and ecosystem services and they do not always have enough knowledge for this new task. Follow-up, evaluation and control systems are also necessary for achieving good value for money.

The ambition, in the medium term, should be to implement a real market for public goods and services. Most CAP expenditures should be redirected to this objective and affected to pillar 2. That will give a long-term legitimacy to the CAP budget.

The relative importance of the EU as an exporter on the world market will probably decrease. Only luxury and high-quality products like Protected Designation of Origin (PDO) wines and cheeses, have good chances to compete in this market. European farmers should maintain or increase their income by producing for local markets in priority and by increasingly selling their products in short-marketing chains. PDO labels and private trademarks can be efficient tools for guaranteeing the combination of local origin, better taste, and the protection of biodiversity and landscape. That requires the consideration of biodiversity in the specifications to ensure a production system that favours biodiversity. Agri-tourism can also be helpful, especially in less favoured areas.

**Improving human health and achieving higher protein independence are objectives that can be partly achieved by a better use of grasslands**, by reducing the importance of monogastric meat consumption compared to beef meat, by producing meat and dairy products on the basis of grass and not on the basis of grain. More grasslands in the UAA will also provide better landscapes and more ecosystem services.

**Food waste reduction should receive more attention**. Policies can in parallel reduce wastes and use them, for instance in animal feeding as sources of protein and energy. Innovative systems are needed for ensuring the respect of hygienic aspects.

**Research and development are essential** for developing new systems that are efficient both in terms of food and ecosystem service productions. Innovation in the bio-economy is though not restricted to biotechnologies. New solutions, combining existing knowledge and techniques, or using new techniques, can be developed at the whole farming system level. Research is notably required to define the economic value of public goods and services in different kinds of ecosystems in arable land, grassland and forest; to study the effects of agricultural techniques and systems on the delivery of these public goods and services; to support the conservation and restoration of public goods and services from the technical and economic points of view. Small and Medium Enterprises (SME) should be more intensively associated with the development of new commercial products and processes (Murphy-Bokern *et al.*, 2011).

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# References

Allen V.G., Batello C., Berretta E.J., Hodgson J., Kothmann M., Li X., McIvor J., Milne J., Morris C., Peeters A. and Sanderson M. (2011) An International Terminology for Grazing Lands and Grazing Animals. *Grass and Forage Science* 66, 2–28.

Beaufoy G. and Marsden K. (2011) *CAP reform 2013 – last chance to stop the decline of Europe's High Nature Value farming?* EFNCP, BirdLife International, Butterfly Conservation Europe, WWF, 35 pp.

Bossuet I., Chambaut H., Le Gall A. and Raison C. (2006) Study of the distribution of nitrogen surplus in experimental dairy farms of the Atlantic Area. Proceedings of the Green Dairy Project Final Seminar, Rennes, December 2006. Institut de l'Elevage, Rennes, France, pp. 67–92.

Bretagnolle V., Villers A., Denonfoux L., Cornulier T., Inchausti P. and Badenhausser I. (2011) Rapid recovery of a depleted population of Little Bustards *Tetrax tetrax* following provision of alfalfa through an agri-environment scheme. *Ibis* 153, 4–13.

Brogan J.C. (1966) Organic carbon in Irish pasture soils. *Irish Journal of Agriculture and Food Research* 5, 169–176.

Cerdan O., Govers G., Le Bissonnais Y., Van Oost K., Poesen J., Saby N., Gobin A., Vacca A., Quinton J., Auerswald K., Klik A., Kwaad F.J.P.M., Raclot D., Ionita I., Rejman J., Rousseva S., Muxart T., Roxo M.J. and Dostal T. (2010) Rates and spatial variations of soil erosion in Europe: A study based on erosion plot data. *Geomorphology* 122, 167–177.

Couvreur S., Hurtaud C., Lopez C., Delaby L. and Peyraud J.L. (2006) The linear relationship between the proportion of fresh grass in the cow diet, milk fatty acid composition, and butter properties. *Journal of Dairy Science* 89, 1956–1969.

Dauber J., Bengtsson J. and Lenoir L. (2006) Evaluating effects of habitat loss and land-use continuity on ant species richness in semi-natural grassland remnants. *Conservation Biology* 20, 1150–1160.

de Sainte-Marie C. (2009) Favoriser la biodiversité par des mesures agri-environnementales à obligation de résultat. Les prairies fleuries du Massif des Bauges (Savoie). INRA ed. Downloadable from: http://www. inra.fr/layout/set/print/presse/mesures\_agri\_environnementales\_avec\_engagement\_de\_resultat\_ecologique. Delgado C. (2005) Rising demand for meat and milk in developing countries: implications for grasslandsbased livestock production. In: McGilloway D.A. (ed.) Grassland: a global resource. Proceedings of the XX IGC meeting, Wageningen Academic Publishers, The Netherlands, pp. 29–39.

Dhiman T.R., Anand G.R., Satter L.D. and Pariza M.W. (1999) Conjugated linoleic acid content of milk from cows fed different diets. *Journal of Dairy Science* 82, 2146–2156.

Dillon P. (2010) Managing European Grasslands to Increase the Sustainability and Competitiveness of Livestock Production Systems. DG Research, MULTISWARD (FP7-244983), Deliverable 4.1, pp. 18.

Donald P.F., Sanderson F.J., Burfield I.J. and van Bommel F.P.J. (2006) Further evidence of continent-wide impacts of agricultural intensification on European farmland birds, 1990–2000. *Agriculture, Ecosystems and Environment* 116, 189–196.

Duckett S.K, Wagner D.G., Yates L.D., Dolezal H.G. and May S.G. (1993) Effects of time on feed on beef nutrient composition. *Journal of Animal Science* 71, 2079–2088.

Duquesne B., Matendo S. and Lebailly Ph. (2006) Profiling food consumption: comparison between USA and EU. USDA and AIEA2 International Meeting "*Competitiveness in Agriculture and in the Food Industry: US and EU perspectives*" – University of Bologna – June 15–16, 2006. Symposium Proceedings, pp. 11. Downloadable from: agriculture.wallonie.be/apps/spip\_wolwin/IMG/pdf/usa.pdf.

European Commission (2003) The meat sector in the European Union. DG Agriculture, 20 pp.

European Commission (2010) *Preparatory study on food waste across EU 27*. Final report. DG Environment Directorate C, 210 pp.

European Commission (2011a) Proposal for a Regulation of the European Parliament and of the Council establishing rules for direct payments to farmers under support schemes within the framework of the common agricultural policy. COM(2011) 625/3, 104 pp.

European Commission (2011b) *Proposal for a Proposal for a Regulation of the European Parliament and of the Council on support for rural development by the European Agricultural Fund for Rural Development (EAFRD).* COM(2011) 627/3, 143 pp.

Godfray H.C.J., Crute I.R., Haddad L., Lawrence D., Muir J.F., Nisbett N., Pretty J., Robinson S., Toulmin C. and Whiteley R. (2010) The future of the global food system. *Phil. Trans. R. Soc. B* 365, 2769–2777. Hart K. and Baldock D. (2011) *Greening the CAP: delivering environmental outcomes through pillar one*. Institute for European Environmental Policy (IEEP), 26 pp.

Janssens I.A., Freibauer A., Schlamadinger B., Ceulemans R., Ciais P., Dolman A.J., Heimann M., Nabuurs G.-J., Smith P., Valentini R. and Schulze E.-D. (2005) The carbon budget of terrestrial ecosystems at country-scale – a European case study. *Biogeosciences* 2, 15–26.

Kearney J. (2010) Food consumption trends and drivers. Phil. Trans. R. Soc. B 365, 2793–2807.

Matzdorf B., Kaiser T. and Rohner M.S. (2008) Developing biodiversity indicator to design efficient agrienvironmental schemes for extensively used grassland. *Ecological Indicators* 8, 256–269.

McGrath D. and Zhang C. (2003) Spatial distribution of soil organic carbon concentrations in grassland of Ireland. *Applied Geochemistry* 18, 1629–1639.

Murphy-Bokern D., Gherghinescu O., Kraigher H., Lazzeri P., Peeters A., Schmid O., Scudamore J., Toepfer S. and Westergaard J. (2011) *Impacts of EU Framework Programmes (2000–2010) and prospects for research and innovation in agriculture, animal health and welfare, and forestry.* Report to the European Commission, 112 pp.

O'Connell K., Humphreys J. and Watson C.J. (2003) Quantification of nitrogen sources for grassland. In: Proceedings of the Fertiliser Association of Ireland, 40, pp. 15–29.

OECD (2011) OECD Health Data 2011. Downloadable from: http://www.oecd.org/document/16/0,2340, en\_2649\_34631\_2085200\_1\_1\_1\_1\_0.0.html.

Oppermann R. (2003) Nature balance scheme for farms – evaluation of the ecological situation. *Agriculture, Ecosystems & Environment* 98, 463–475.

Parfitt J., Barthel M. and Macnaughton S. (2010) Food waste within food supply chains: quantification and potential for change to 2050. *Phil. Trans. R. Soc. B* 365, 3065–3081.

Peeters A. (2008) Public demands on intensive grassland systems and agri-environmental policies of OECD members. *Multifunctional Grassland in a Changing World* 1, 15–31.

Peeters A. (2009) Importance, evolution, environmental impact and future challenges of grasslands and grassland-based systems in Europe. *Grassland Science* 55, 113–125.

Peeters A. (2010) Importance and evolutions of grasslands and grassland-based farming systems in Europe. Socio-economic and political driving forces of these evolutions. DG Research, MULTISWARD (FP7-244983), Deliverable 5.1, 64 pp.

Plantureux S., Peeters A. and McCracken D. (2005) Biodiversity in intensive grasslands: effect of management improvement and challenges. *Agronomy Research* 3, 153–164.

Rule D.C., Broughton K.S., Shellito S.M. and Maiorano G. (2002) Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk and chicken. *Journal of Animal Science* 80, 1202–1211. Sapir A., Aghion Ph., Bertola G., Hellwig M., Pisani-Ferry J., Rosati D., Viñals J. and Wallace H. (2003) *An agenda for a growing Europe. Making the EU Economic System Deliver*. Report of an Independent High-Level Study Group established on the initiative of the President of the European Commission, 173 pp. Simopolous A.P. and Robinson J. (1999) *The Omega Diet*. New York, Harper Collins.

Simopoulos A.P. (2002) The Importance of the Ratio of Omega-6/Omega-3 Essential Fatty Acids. *Biomed. Pharmacother* 56, 365–379.

Swolfs S. (2011) Evolution of animal feed import in the EU: possible ecological impacts. Universiteit Antwerpen, Master in de Milieuwetenschap, Academiejaar 2010–2011, 92 pp.

Thornton P.K. (2010) Livestock production: recent trends, future prospects. *Phil. Trans. R. Soc. B* 365, 2853–2867.

Van Zeijts H. et al. (2011) Greening the Common Agricultural Policy: impacts on farmland biodiversity on a EU scale. The Hague, PBL Netherlands Environmental Assessment Agency, 61 pp.

Veen P., Molnár Z., Pärtel M. and Nagy S. (2001) Grassland ecosystems in Central and Eastern Europe. Prepared in the framework of the High Level Conference on EU Enlargement 'The relation between agriculture and nature management', 19 pp.

Vleeshouwers L.M. and Verhagen A. (2001) CESAR: a model for carbon emission and sequestration by agricultural land use. Report 36, Plant Research International, Wageningen, 27 pp.

Von Witzke H. and Noleppa S. (2010) *EU Agricultural Production and Trade: Can More Efficiency Prevent Increasing 'Land-Grabbing' Outside Of Europe?* Agripol, OPERA – Research Centre, 36 pp.

Walker K.J., Stevens P.A., Stevens D.P., Mountford J.O., Manchester S.J. and Pywell R.F. (2005) The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation* 119, 1–18.

Westhoek H., Rood T., van den Berg M., Janse J., Nijdam D., Reudink M. and Stehfest E. (2011) *The Protein Puzzle. The consumption and production of meat, dairy and fish in the European Union*. The Hague, PBL Netherlands Environmental Assessment Agency, 218 pp.

Wittig B., Kemmermann A.R. gen. and Zacharias D. (2006) An indicator species approach for resultorientated subsidies of ecological services in grasslands: a study in Northwestern Germany. *Biological Conservation* 133, 186–197.

World Health Organization (WHO) (2012) *Obesity and overweight*. Downloadable from: http://www.who. int/mediacentre/factsheets/fs311/en/index.html.

WRAP (2009) Household food and drink waste in the UK. Banbury, UK.

WRAP (2010) A review of waste arising in the supply of food and drink to UK households. Banbury, UK.

# Session 1

# **Grassland-based farming systems**

# Agronomic value and provisioning services of multi-species swards

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# Abstract

The Millenium Ecosystem Assessment provides a new framework for investigating the multifunctionality of grasslands while the challenges of scarcity underlined by the last report of the Standing Committee for Agricultural Research stress the need for innovations towards more efficient production systems. Multi-species grasslands will be a main input to improve energy and water efficiencies of forage and animal production. The present paper reviewed biomass production in multispecies swards and analysed the role of species number, functional diversity, species abundance and species identity. It also focussed on the interaction with agronomic conditions and especially fertilization.

Beyond biomass production, other ecosystem services provided by multispecies sown grasslands were investigated, i.e. weed control during establishment and nitrogen losses by animals as a consequence of the sugar/protein ratio.

In order to better valorize these possible services, it is necessary to better understand the underlying mechanisms. Competition, facilitation and complementarity explain most of the ecosystem services. However, the species composition of the plant community is the key driver. Based on the processes of habitat filtering and niche differentiation, it is possible to combine species diversity and genetic diversity in a comprehensive and integrated concept, showing how an interdisciplinarity approach of ecology, agronomy and plant genetics will provide innovations.

Keywords: functional diversity, genetic diversity, ecological concepts, overyielding, environmental benefits, sown grassland

# Introduction

Agriculture is changing in line with the major society drivers. Governed by a strong and committed European policy, farming has been a highly regulated business. To cope with this, the most striking development during the past years is the very fast expansion of agricultural enterprises both in terms of means of production, technology application, management and alliances. And this also holds true for ruminant production and the associated grassland management. While this evolution is going on, scientists and policy makers already think way ahead of present evolutions. A striking example is the latest report of the Standing Committee of Agricultural Research (SCAR) (Freibauer *et al.*, 2011). The report clearly underlines the challenge of scarcity of resources (water, energy, land, but also human labour). It proposes to move away from the existing paradigm of productivity and replace it by the paradigm of efficiency. The report states "Scientific advance has the potential to bring forward agro-ecosystems that are both productive, respectful for ecosystems and resource saving. Demand increases need to be mitigated through behavioral changes, the internalization of environmental externalities and appropriate governance structures".

The SCAR report suggests that consumer-driven and technology-driven pathways are building blocks of the transition. This second group is highly relevant for grassland production, where it is possible to close **yield gap** through ecological intensification, **resilience gap** through diversification and implementation of more resilient agri-food production systems. Multispecies

grasslands may clearly contribute to close these gaps. The SCAR report also underlines that the transition also includes **innovation gap** for a quick adoption of research results by the farmers and the **socio-technology gap** meaning that farmers and industries have to accept risks associated with transition. In the case of multispecies grasslands, these last two gaps are very challenging. Closing the socio-technology gap requires to overcome the risk aversion of farmers (Feder, 1980).

It is proposed in this paper to analyze the potential of multispecies grasslands to get a high agronomic performance, in the new paradigm of efficiency, i.e. providing multiple ecosystem services as defined by the Millennium Ecosystem Assessment (Reid, 2005)

Before reviewing the potential agronomic and environmental values of multispecies grasslands, it is relevant to reassess how they were considered in the past and how new approaches and knowledge are opening new prospects.

One of the first reports of benefits of multispecies grasslands was given by Charles Darwin himself (1872) who stated that "*it was experimentally demonstrated that if a plot of land was sown with a single grass species and a similar plot with a mixture of various genus of grasses, then a higher quantity of dry forage was produced in the second plot than in the first*'. He actually reported farm-scale works run by the Duke of Bedford some years earlier, although little information was given on the soil, climate, stocking rate and sheep grazing management.

Sowing grasslands with a high species diversity has long been practiced in Europe and worldwide. This was due to the difficulty of buying seeds for pure species and to local observations and know-how by farmers.

But, the Forage Revolution deeply changed the interest of agronomists in species diversity in grasslands for three reasons. The first reason was clearly the adoption of high nitrogen fertilization, with a 'poorer' performance of multispecies grasslands under such practices. In a study in New-Zealand, Ruz-Jerez et al. (1991) compared under sheep grazing a ryegrass sward fertilized with 400 N ha<sup>-1</sup> year<sup>-1</sup>, a multispecies sward with 23 grass, legumes and dicots species and a perennial ryegrass – white clover mixture, with mean yields of 16.5, 15 and 12 t DM ha<sup>-1</sup> respectively. The second reason was the adoption of forage crops developed for cutting and short duration. The last reason was clearly the reduction of risk associated with management, which was long considered to be a drawback of multispecies swards. This was for instance underlined by Blaser et al. (1952) who quoted that complex mixtures of grass and legumes had little future because of the unpredictable fate of competition among seedlings during the establishment phase or later among plants. In the new paradigm of efficiency which makes it possible to meet environmental expectations, these driving forces are changing. The first one is deeply modified due to an upcoming scarcity of resources. The last two must find an answer through modified practices and a better understanding of the underlying mechanisms of multispecies swards.

Emergence of community ecology as a scientific discipline deeply modifies the scientific context, provides new concepts and makes it possible to provide breakthroughs and innovations, especially when working in interdisciplinarity with agronomists and geneticists. In the present paper, we will 1) review relationship between species diversity and agronomic value, and 2) investigate the underlying mechanisms which make it possible to envisage future prospects.

1. Relation between species diversity and agronomic value: what is new in the literature?

The scientific literature shows, over the last years, a huge increase in the number of papers focusing on multispecies ecosystems, and especially grassland ecosystems, this is for instance demonstrated by the large number of citations of the keystone paper published by Hector *et al.* (1999), which had reached 802 at the date of writing the present paper. We will not review all the literature but focus on four particular points which are the role of (i) species and (ii) functional diversity, (iii) the question of species identity and (iv) the importance of soil and climate conditions and agronomic practices. Most papers only consider biomass production.

# 1.1. Role of species diversity

Most papers report a positive relationship between species diversity and biomass production. The first major report is the paper by Hector *et al.* (1999) based upon 8 sites in Europe, with species number ranging from 1 to 32 in one Swiss location, species being grasses, legumes and dicots. A general trend was observed with an increasing primary productivity with an increasing number of species (Figure 1). However, for most sites this study was carried out on a single year and there was one species which yielded as much as the most productive multispecies swards.



Figure 1. Relationship between number of sown species and biomass production in eight sites in Europe (from Hector *et al.*, 1999)

This first study was followed and supported by numerous other ones. We may quote the study run by Guo *et al.* (2006) with locally adapted populations of grassland species, in four sites with contrasting agronomic potential, over three years (Figure 2). The general trend of benefit with species diversity was observed over all sites and years but the highest productivities were often achieved with intermediate species diversity.

The study by Kirwan *et al.* (2007) was based upon 28 sites belonging to 4 types of soil and climate conditions in Europe, where 11 combinations of grass and legumes were tested, these combinations being obtained with four species (two grasses and two legumes) adapted to the environments. This research clearly underlines the positive interaction between grass and legumes, but also grass-grass and legume-legume interactions, these last two types of interactions being less anticipated (Figure 3). This research also pointed out the importance of evenness.



Figure 2. Relationship between species diversity and grassland productivity during three years and four sites in North Dakota (from Guo *et al.*, 2006)

Many more papers illustrate this positive relationship under the present weather conditions. De Boeck *et al.* (2008) also found this relationship under simulated conditions affected by climate changes. Interestingly, very few papers found a negative relationship between species diversity and productivity, such as Grace *et al.* (2007).

Beyond the short-term relationship, its stability over years and conditions is critical. Although early theoretical discussions established the axiom that diverse, complex ecological communities are the most stable (MacArthur, 1955), this is highly debated as in Hector (2006). This was approached in the work of Guo *et al.* (2006) and investigated in details by Sanderson (2010) on the basis of 175 mixtures of grasses and legumes in 3 to 6 year experiments in the USA. No consistent relationship between yield stability and measures of diversity was evidenced. It was pointed out that species identity and assemblage could be more important than simply the number of species. This would be relevant with the functional redundancy hypothesis (Johnson, 2000).



Figure 3. Relationship between species diversity and agronomic value of multispecies swards in four types of soil and climate conditions in Europe. ME: Mild European conditions, NE: Nordic Europe, MM: Mild Mediterranean conditions, DM: Dry Mediterranean conditions (from Kirwan *et al.*, 2007).

# 1.2. Role of species identity and abundance

Importance of identity of the dominant species was underlined (Suter *et al.*, 2010), and proved to be density-dependent. This was explained by their ability of occupy aboveground space and thus capture incident photosynthetic radiation (Lorentzen *et al.*, 2008). Moreover, it was shown that their identity was shaping the species composition of the swards (Miles and Knops, 2009).

Pontes *et al.* (2007a) underlined that, beyond diversity, the absolute values of some leaf traits, such as dry matter and nitrogen contents, were essential to determine not only the agronomic value of grassland swards but also the fate of the species within the swards. Surault *et al.* (2008) and Deak *et al.* (2007) in France and US respectively showed that the identity of dominant species was a key factor for biomass production. In both studies run in fertile environments with high summer temperature, cocksfoot and tall fescue appeared to be the key species to achieve high yield in multispecies swards.

We suggest that the concept of essentiality could be usefully and easily applied to grasslands. This concept was defined on algae by Zhang and Zhang (2007). The essentiality index of a species S could be calculated as the relative difference between the yield of a community and the yield of a community with all the same species but S.

# 1.3. Role of functional diversity

More than the number of species, functional diversity has a beneficial effect on biomass production as shown by Hector *et al.* (1999), Jiang *et al.* (2007), Kirwan *et al.* (2007) and Lanta and Leps (2006).

Diaz and Cabido (2001) identified functional groups as groups of species which share common responses to biotic and abiotic factors and exert similar effects on the ecosystem functioning. This definition underlines the importance of the response/effect traits taken into account to measure the response and effect when defining the groups. The function of symbiotic nitrogen fixation clearly separates grass and legumes, but the limits are more difficult to establish within each of these groups, as continuous variations are described among species for most functional traits (Duru *et al.*, 2009; Pontes *et al.*, 2007a; 2007b). The

study run by Poozesh *et al.* (2007) illustrated the relevance to take into account traits of resistance to abiotic stress, aluminium tolerance in this study, to structuring the functional groups. Within grasses, contrasting water deficit responses between tall fescue and cocksfoot make it possible to separate these two grass species in two different functional groups (Gastal and Durand, unpublished). Mixtures of cocksfoot and tall fescue exhibit overyield-ing especially in dry environments and this may be explained by the increased functional diversity for water deficit responses.

The functional groups allow integrating function of species among different groups and avoid response/effect redundancy between species in the same community. In consequence, with this species classification, we can understand the species relationships and grassland functioning. However, the relative abundance of species and functional groups plays a key role. Indeed, the response/effect of species in community may be connected to its relative abundance (Fridley, 2002).

## 1.4. Management regime plays a key role

Environmental conditions will determine presence of the various species present in the plant community. They induce a habitat filtering (Podani, 2009) that selects only adapted species. However, these environmental conditions can also drive the structure of selected species. In grassland, conditions induced by the farming practices such as fertilization (resource availability) or grazing management and the interaction between soil, climate conditions and species composition, will play a key role and could well alter a diversity – productivity relationship.

Grazing has probably the most important effect for the structure of the grassland community (Rook and Tallowin, 2003) and the diversity – productivity relationship. Indeed, it will influence the sward structure and composition in three ways:

- a) Height and regularity of defoliation, in relation with the animal species.
- b) Heterogeneities by patch. Grazing will generate variation in sward height, with even some areas with bare soils either due to overgrazing or during dry periods. There will also be fertility heterogeneities due to dung and urine deposition.
- c) Dissemination of seeds. It was shown that grazing animals are able to move seeds either on their skin (exozoochory) or through their guts (endozoochory) (Cosyns *et al.*, 2006; Pakeman and Small, 2009).

The most extreme variation is observed when grazing is stopped, leading to a strong change of botanical composition of grasslands with a reduction in species diversity and predominance of some vegetatively propagated aggressive species (Amiaud *et al.*, 2008).

The farming practices are not unique environmental pressures on grassland, the resource availability effect is important to community structure.



Figure 4. Decline in species diversity is related to the magnitude of production increase on plots after N fertilization. SDn and SDc, species density (number of species per area) in fertilized and control plots, respectively; ANPPn and ANPPc, above-ground net primary production in fertilized and control plots, respectively. Open symbols indicate marshes (from Suding *et al.*, 2005)

Plant diversity was negatively correlated with productivity in suitable environments (Rajaniemi, 2003) and declined with an increasing productivity in most environments after N fertilization (Figure 4) (Suding *et al.*, 2005). These results may be explained with different mechanisms as presented in detail in section 3.

2. Underlying mechanisms. Why do multi-species grassland communities display overvielding? What is the expected impact of genetic diversity in the mechanisms? A key question in ecology concerns the distribution and abundance of species, i.e. community assembly. Ecological theory has proposed a view of community assembly as a series of nested processes, which leads to a species coexisting at a given site (Diamond, 1975; Weiher and Keddy, 1999). These processes, which integrate interactions among species/abiotic environment and species/species, determine distribution and abundance of species. Among processes that contribute to the assembly of communities, *habitat filtering* (Figure 5) (Podani, 2009) and *niche differentiation* (MacArthur and Levins, 1967; Stubbs and Wilson, 2004) are identified as key processes. The theory of "habitat filtering" is that community composition results from biotic and abiotic filters that select species with similar characteristics (Grime, 2006). For example, in Mediterranean environment, species without traits that allow survival under water deficit are excluded. Conversely, niche differentiation or competitive exclusion, which relates to functional diversity of species, is based on functional complementarity (Silvertown, 2004), which implies a decrease in interspecific competition (Gross et al., 2007), can reduce similarity between co-existing species (Pacala and Tilman, 1994). If two species take up the same resource, in the same way, at the same moment and at the same site, the co-existing probability of these species may be very low, due to competition. In contrast, species displaying different functional traits and exhibiting complementarity



(use of a same resource at different times or sites and use of different resources for a similar physiological process) or facilitation (presence of one species increases the probability of presence of the second one) will co-exist. But we must consider the multidimensional characteristic of species niches, and the global interaction between species is the result of interaction on different axes (function of resources) of niche.

On the basis of these ecological theories, ecosystem services of multi-species grassland communities may be explained as follows: while grassland species of temporary grasslands have relatively similar trait values, for example as cutting tolerance; a spatial and/or temporal differentiation of

Figure 5. Consequences of habitat filtering in presence of genetic diversity on species diversity in niches (from Jung *et al.*, 2010)

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resource needs, through differentiation of functional traits, may increase use efficiency of resources that leads to improve species co-existence and community productivity (Hector *et al.*, 1999; Lanta and Leps, 2006; Kirwan *et al.*, 2007; Jiang *et al.*, 2007). We note that the functional interactions between species may be a view of competition gradient from strong competition (resulting in species exclusion) to facilitation (advantage of co-existence between two or more species). The relationship between resources availability and intensity of the impact of the niche differentiation theory is evident. The role of this mechanism increases with the limitation of resources (Nyfeler, 2009). This point underlines the benefit of using multi-species grassland, and particularly grass-legume mixtures, which represent a good example of facilitation interactions.

Grassland diversity can be approached at various levels of complexity (McGill *et al.*, 2007), ranging from species diversity to genetic diversity. The majority of studies exploring diversity and community dynamics to date have focused on species and functional diversity and interspecific interactions (Lortie *et al.*, 2004; Craine, 2005; Brooker *et al.*, 2008). However, recent studies highlighted importance of intraspecific variation (Booth and Grime, 2003; Fridley *et al.*, 2007; Vellend and Litrico, 2008) for community assembly (Jung *et al.*, 2010) and community services. As stated in section 1, studies of grass-legume mixtures have found higher yield compared to that of each species in monospecific swards (white clover and ryegrass: Turkington and Jolliffe, 1996; Charles and Lehmann, 1989). The consequences of species diversity on forage quality are matters of debates, the dilution effect being the main driver for the major components of forage quality such as protein content and digestibility, while the presence of secondary compounds such as tannins may induce feed interactions among forages (Huyghe *et al.*, 2008).

Nevertheless, differences in "compatibility" of varieties of clover and ryegrass have been demonstrated (Evans, 1989), confirming the idea that agronomic traits and genetic composition of varieties may play a key role in multispecies mixtures. In this context, mechanisms involved in overyielding of multispecies grasslands should be studied to integrate different scales. The transfer of niche differentiation mechanisms from interspecific scale to the genotype scale is necessary. This approach may explain how the genetic diversity is shaped in natural grasslands. This approach based on the modes of genotype functioning (i.e. on the characteristics involved in the fitness and productivity of genotypes) impacts plant breeding that has the potential to enhance efficiency and benefits derived from sown grasslands. But selecting varieties based on their ability to coexist in mixtures presents logistic difficulties (large numbers of combinations to test, confounding influences of the environment). The most realistic solution to create such varieties is the coupling of breeding with a mechanistic approach based on interactions within the sward; this has the advantage of leading to general results that help define variety ideotypes.

## **Discussion – conclusions**

This literature review of the relationship between species diversity in grasslands and ecosystem services shows that a positive relationship is frequently found with biomass production. However, there are some cases where it does not exist, that the various components of diversity play a role, especially species identity in relation with functional characteristics and it depends on environmental conditions, especially management and N fertilization. Interestingly, very few cases of a negative relationship were reported. The understanding of the underlying physiological and ecological mechanisms explains this range of responses. This review makes it possible to identify new questions to research and to identify practical recommendations. It is essential to understand the mechanisms, especially those related to interactions between plants and to functional traits and groups. Explicit models, supporting the present theoretical models would be a breakthrough. Beyond the relationship between species diversity and within-species diversity, it could be questioned how within-species diversity could be further exploited.

Several practical recommendations can be produced from this review. The first one deals with the choice of the species to be used in multispecies grasslands. It is important to optimize functional diversity, combining species and genotypes with contrasting plant heights, phenologies and resource capture strategies, thus increasing the occurrence of complementarity and facilitation. This clearly legitimates the frequent grass and legume mixtures, but also the attempts to include other dicots, such as chicory (Haring *et al.*, 2008). But this review clearly underlines the need to screen species, and varieties, well adapted to the local environments with a strong growth, although this questions the long term equilibrium of the plant community, and the interaction with the fertility.

A technical question arises from the possibility to ensure the turnover of the plants within the swards. The agronomic practices to handle the species diversity via seedling recruitment are difficult to tune, although the over-seeding techniques open new prospects and they are under-used, including for permanent grasslands. A better understanding of the mechanisms, including at the seedling stage (Korner *et al.*, 2008), will likely provide elements for a better and more effective management of over-seeding.

Finally, this review offers a new orientation for agriculture and multispecies grasslands to meet the challenge of scarcity. Indeed, increasing species diversity and exploiting genetic diversity may be beneficial to productivity and to environment preservation. But it has for sure a positive impact on the other biodiversity compartments, at field or landscape scales via trophic chains (Duffy *et al.*, 2007). Thus, even when the consequences on biomass production and quality are limited, benefits for biodiversity and potentially for other ecosystem services fully justify the use of multispecies grasslands and adapted management practices. However, it is important to consider that this must be achieved without extra work load or uncertainties for the farmers.

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# References

Amiaud B., Touzard B., Bonis A. and Bouzille J.B. (2008) After grazing exclusion, is there any modification of strategy for two guerrilla species: *Elymus repens* (L.) Gould and *Agrostis stolonifera* (L.)? *Plant Ecology* 197, 107–117.

Blaser R.E., Skrdla W.H. and Taylor T.H. (1952) Ecological and physiological factors in compounding seed mixtures. *Advances in Agronomy* 4, 179–218.

Booth R.E. and Grime J.P. (2003) Effects of genetic impoverishment on plant community diversity. *Journal of Ecology* 91, 721–730.

Brooker R.W., Maestre F.T., Callaway R.M., Lortie C.L., Cavieres L.A., Kunstler G., Liancourt P., Tielborger K., Travis J.M.J., Anthelme F., Armas C., Coll L., Corcket E., Delzon S., Forey E., Kikvidze Z., Olofsson J., Pugnaire F., Quiroz C.L., Saccone P., Schiffers K., Seifan M., Touzard B. and Michalet R. (2008) Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology* 96, 18–34. Charles J.P. and Lehmann J. (1989) Intérêt des mélanges de graminées et de légumineuses pour la production fourragères suisse ? *Fourrages* 199, 311–320.

Cosyns E., Bossuyt B., Hoffmann M., Vervaet H. and Lens L. (2006) Seedling establishment after endozoochory in disturbed and undisturbed grasslands. *Basic and Applied Ecology* 7, 360–369.

Craine J.M. (2005) Reconciling plant strategy theories of Grime and Tilman. Journal of Ecology 93, 1041–1052.

De Boeck H.J., Lemmens C., Zavalloni C., Gielen B., Malchair S., Carnol M., Merckx R., Van den Berge J., Ceulemans R. and Nijs I. (2008) Biomass production in experimental grasslands of different species richness during three years of climate warming. *Biogeosciences* 5, 585–594.

Deak A., Hall M.H., Sanderson M.A. and Archibald D.D. (2007) Production and nutritive value of grazed simple and complex forage mixtures. *Agronomy Journal* 99, 814–821.

Diamond J. M. (1975) Assembly of species communities. In: Cody M. L. and Diamond J.M. (eds). Ecology and evolution of communities. Harvard University Press, Cambridge, Massachusetts, USA. pp. 342–444

Diaz S. and Cabido M. (2001) Vive la difference: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution* 16, 646–655.

Duffy J.E., Cardinale B.J., France K.E., McIntyre P.B., Thebault E. and Loreau M. (2007) The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology Letters* 10, 522–538.

Duru M., Adam M., Cruz P., Martin G., Ansquer P., Ducouytieux C., Jouany C., Theau J.P. and Viegas J. (2009) Modelling above-ground herbage mass for a wide range of grassland community types. *Ecological Modelling* 220, 209–225.

Evans D. (1989) Variation in grass yield in cover grass mixure. *Report of the Welsh Plant breeding Station*, 14 p.

Feder G. (1980) Farm-size, risk aversion and the adoption of new technology under uncertainty. *Oxford Economic Papers-New Series* 32, 263–283.

Freibauer A., Mathijs E., Brunori G., Daminanova Z., E.F., Girona I., Gomis J., O'Brien L. and Treyer S. (2011) Sustainable food consumption and production in a resource constrained world. SCAR. http://ec.europa.eu/research/agriculture/scar/pdf/scar\_feg3\_final\_report\_01\_02\_2011.pdf

Fridley J.D. (2002) Resource availability dominates and alters the relationship between species diversity and ecosystem productivity in experimental plant communities. *Oecologia* 132, 271–277.

Fridley J.D., Stachowicz J.J., Naeem S., Sax D.F., Seabloom E.W., Smith M.D., Stohlgren T.J., Tilman D. and Von Holle B. (2007) The invasion paradox: Reconciling pattern and process in species invasions. *Ecology* 88, 3–17.

Grace J.B., Anderson T.M., Smith M.D., Seabloom E., Andelman S.J., Meche G., Weiher E., Allain L.K., Jutila H., Sankaran M., Knops J., Ritchie M. and Willig M.R. (2007) Does species diversity limit productivity in natural grassland communities? *Ecology Letters* 10, 680–689.

Grime J.P. (2006) Trait convergence and trait divergence in herbaceous plant communities: mechanisms and consequences. *Journal of Vegetation Science* 17, 255–260.

Gross N., Suding K.N., Lavorel S. and Roumet C. (2007) Complementarity as a mechanism of coexistence between functional groups of grasses. *Journal of Ecology* 95, 1296–1305.

Guo Q.F., Shaffer T. and Buhl T. (2006) Community maturity, species saturation and the variant diversity-productivity relationships in grasslands. *Ecology Letters* 9, 1284–1292.

Haring D.A., Scharenberg A., Heckendorn F., Dohme F., Luscher A., Maurer V., Suter D. and Hertzberg H. (2008) Tanniferous forage plants: Agronomic performance, palatability and efficacy against parasitic nematodes in sheep. *Renewable Agriculture and Food Systems* 23, 19–29.

Hector A. (2006) Overyielding and stable species coexistence. New Phytologist 172, 1-3.

Hector A., Schmid B., Beierkuhnlein C., Caldeira M.C., Diemer M., Dimitrakopoulos P.G., Finn J.A., Freitas H., Giller P.S., Good J., Harris R., Hogberg P., Huss-Danell K., Joshi J., Jumpponen A., Korner C., Leadley P.W., Loreau M., Minns A., Mulder C.P.H., O'Donovan G., Otway S.J., Pereira J.S., Prinz A., Read D.J., Scherer-Lorenzen M., Schulze E.D., Siamantziouras A.S.D., Spehn E.M., Terry A.C., Troumbis A.Y., Woodward F.I., Yachi S. and Lawton J.H. (1999) Plant diversity and productivity experiments in European grasslands. *Science* 286, 1123–1127.

Huyghe C., Baumont R. and Isselstein J. (2008) Plant diversity in grasslands and feed quality. *Grassland Science in Europe* 13, 375–386.

Jiang X.L., Zhang W.G. and Wang G. (2007) Effects of different components of diversity on productivity in artificial plant communities. *Ecological Research* 22, 629–634.

Johnson K.H. (2000) Trophic-dynamic considerations in relating species diversity to ecosystem resilience. *Biological Reviews* 75, 347–376.

Jung V., Violle C., Mondy C., Hoffmann L. and Muller S. (2010) Intraspecific variability and trait-based community assembly. *Journal of Ecology* 98, 1134–1140.

Kirwan L., Luescher A., Sebastia M.T., Finn J.A., Collins R.P., Porqueddu C., Helgadottir A., Baadshaug O.H., Brophy C., Coran C., Dalmannsdottir S., Delgado I., Elgersma A., Fothergill M., Frankow-Lindberg B.E., Golinski P., Grieu P., Gustavsson A.M., Hoglind M., Huguenin-Elie O., Iliadis C., Jorgensen M., Kadziuliene Z., Karyotis T., Lunnan T., Malengier M., Maltoni S., Meyer V., Nyfeler D., Nykanen-Kurki P., Parente J., Smit H.J., Thumm U. and Connolly J. (2007) Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology* 95, 530–539.

Korner C., Stocklin J., Reuther-Thiebaud L. and Pelaez-Riedl S. (2008) Small differences in arrival time influence composition and productivity of plant communities. *New Phytologist* 177, 698–705.

Lanta V. and Leps J. (2006) Effect of functional group richness and species richness in manipulated productivity-diversity studies: a glasshouse pot experiment. *Acta Oecologica-International Journal of Ecology* 29, 85–96.

Lorentzen S., Roscher C., Schumacher J., Schulze E.D. and Schmid B. (2008) Species richness and identity affect the use of aboveground space in experimental grasslands. *Perspectives in Plant Ecology Evolution and Systematics* 10, 73–87.

Lortie C.J., Brooker R.W., Choler P., Kikvidze Z., Michalet R., Pugnaire F.I. and Callaway R.M. (2004) Rethinking plant community theory. *Oikos* 107, 433–438.

MacArthur R. (1955) Fluctuations of animal populations and a measure of community stability. *Ecology* 36, 533–536.

MacArthur R.H. and Levins R. (1967) The limiting similarity, convergence, and divergence of coexisting species. *American Naturalist* 101, 377–385.

McGill B.J., Etienne R.S., Gray J.S., Alonso D., Anderson M.J., Benecha H.K., Dornelas M., Enquist B.J., Green J.L., He F.L., Hurlbert A.H., Magurran A.E., Marquet P.A., Maurer B.A., Ostling A., Soykan C.U., Ugland K.I. and White E.P. (2007) Species abundance distributions: moving beyond single prediction theories to integration within an ecological framework. *Ecology Letters* 10, 995–1015.

Miles E.K. and Knops J.M.H. (2009) Grassland compositional change in relation to the identity of the dominant matrix-forming species. *Plant Ecology & Diversity* 2, 265–275.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Lüscher A. (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology* 46, 683–691

Pacala S.W. and Tilman D. (1994) Limiting similarity in mechanistic and spatial models of plant competition in heterogeneous environments. *American Naturalist* 143, 222–257.

Pakeman R.J. and Small J.L. (2009) Potential and realised contribution of endozoochory to seedling establishment. *Basic and Applied Ecology* 10, 656–661.

Podani J. (2009) Convex hulls, habitat filtering, and functional diversity: mathematical elegance versus ecological interpretability. *Community Ecology* 10, 244–250.

Pontes L.D.S., Soussana J.F., Louault F., Andueza D. and Carrere P. (2007a) Leaf traits affect the aboveground productivity and quality of pasture grasses. *Functional Ecology* 21, 844–853.

Pontes L.D.S., Carrere P., Andueza D., Louault F. and Soussana J.F. (2007b) Seasonal productivity and nutritive value of temperate grasses found in semi-natural pastures in Europe: responses to cutting frequency and N supply. *Grass and Forage Science* 62, 485–496.

Poozesh V., Cruz P., Choler P. and Bertoni G. (2007) Relationship between the Al resistance of grasses and their adaptation to an infertile habitat. *Annals of Botany* 99, 947–954.

Rajaniemi T.K. (2003) Explaining productivity-diversity relationships in plants. Oikos 101, 449-457.

Reid W.A. (ed.), 2005. Living beyond our means. Natural assets and human well-being. Millenium Ecosystem Assessment, 28 p. http://www.maweb.org/documents/document.429.aspx.pdf.

Rook A.J. and Tallowin J.R.B. (2003) Grazing and pasture management for biodiversity benefit. *Animal Research* 52, 181–189.

Ruz-Jerez B.E., Ball P.R., White R.E. and Gregg P.E.H. (1991) Comparison of a herbal ley with a ryegrasswhite clover pasture and pure ryegrass sward receiving fertilizer nitrogen. *Proceedings of the New Zealand Grassland Association* 53, 225–230.

Sanderson M.A. (2010) Stability of production and plant species diversity in managed grasslands: A retrospective study. *Basic and Applied Ecology* 11, 216–224.

Silvertown J. (2004) Plant coexistence and the niche. Trends in Ecology & Evolution 19, 605-611.

Stubbs W.J. and Wilson J.B. (2004) Evidence for limiting similarity in a sand dune community. *Journal of Ecology* 92, 557–567.

Suding K.N., Collins S.L., Gough L., Clark C., Cleland E.E., Gross K.L., Milchunas D.G. and Pennings S. (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences of the United States of America* 102, 4387–4392.

Surault F., Veron R. and Huyghe C. (2008) Forage production of pasture mixtures and of associations with various initial specific diversities. *Fourrages* 194, 161–174.

Suter M., Ramseier D., Connolly J. and Edwards P.J. (2010) Species identity and negative density dependence lead to convergence in designed plant mixtures of twelve species. *Basic and Applied Ecology* 11, 627–637. Turkington R. and Jolliffe P. (1996) Using response function models to study the competitive interactions of *Trifolium repens* and *Lolium perenne* in mixtures: short-term and long-term analysis. *Journal of Ecology* 84, 563–571.

Vellend M. and Litrico I. (2008) Sex and space destabilize intransitive competition within and between species. *Proceedings of the Royal Society B: Biological Sciences* 275, 1857.

Weiher E. and Keddy P.A. (1999) Ecological assembly rules:perspectives, advances, retreats. Cambridge University Press, Cambridge, UK.

Zhang Q.G. and Zhang D.Y. (2007) Consequences of individual species loss in biodiversity experiments: An essentiality index. *Acta Oecologica-International Journal of Ecology* 32, 236–242.

# Grassland for sustainable animal production

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# Abstract

Ruminant production is an important function of grasslands but it also interacts positively and negatively with provision of grassland's ecosystem services and the wider environment. While pasture and grass silage are relatively cheap high quality feeds, they are limited in their ability to meet the nutritional needs of productive ruminants. The provision of supplements to correct nutrient imbalances is described, as are the benefits and limitations of extensively managed or semi-natural pastures and of legumes in animal production. The efficiency with which human edible energy and protein are utilised as a feed is highlighted for a range of animal production systems to help decision making. Minimising emissions from ruminant production systems is described, focusing on transmission efficiency at emission spots, especially in the N and P cycles and on greenhouse gases (GHGs), especially methane and nitrous oxide. Carbon (C) footprints are used to describe relative sustainability and efficiency of systems. The interaction between ruminant production and ecosystem services including C sequestration and grassland biodiversity is discussed. While there is scope to improve ruminant production, with high quality grass and forage playing a key role, some trade-offs in mitigating environmental impacts need to be explored further.

Keywords: forage, protein, energy, nutrient cycles, greenhouse gases, carbon footprints

# Introduction

Although ruminant production is only one of a range of functions of European grassland, it is highly important in providing food and maintaining food security. It also plays a vital part in the management of grassland to fulfil other functions, primarily ecosystem services, including maintenance of habitats for biodiversity, and provision of storage (e.g. water and carbon), land stabilisation, and recreation/landscape.

While Europe (i.e. the EU27 countries) is self sufficient in milk, and pig and poultry meat, it is 5 and 20% short of self sufficiency in beef and sheep (and goat) meat, respectively (Hemme, 2010; 2011; Copa-Cogeca, 2009). Achieving and maintaining self sufficiency in food supply should be considered as an integral component of sustainable grassland management as it meets social and economic sustainability criteria while its potential to compromise environmental objectives should be minimised. As ruminants are capable of converting energy and protein that cannot be readily used by most non-ruminants and humans into human edible forms of these dietary constituents, they have a unique role to play in food provision and security. However, grassland production systems often include the purchase of feed and fertilizers or growing of forage crops to increase animal production, leading to unwelcome environmental contaminants (emissions) such as nitrate, ammonia and phosphate and greenhouse gases (GHGs) including nitrous oxide and methane. Purchased feeds include food that could directly be used for human consumption or grown in arable areas that could produce human food directly. Intensiveness may compromise other ecosystem services provided by grassland, such as destruction of habitat and reducing biodiversity.

This paper discusses the drive to produce ruminant products to become or maintain self sufficiency, while minimising environmental damage and making a positive contribution to provision of grassland's ecosystem services.

# **Optimising use of forage**

Thirty three percent of EU27's agricultural area is classified as permanent grassland ranging from temporary grassland not included in a rotation to semi-natural grassland. Throughout the continent of Europe, average annual permanent grassland productivity varies from less than 1.5 Mg DM ha<sup>-1</sup> annum<sup>-1</sup> in the Mediterranean region to about 10 Mg DM ha<sup>-1</sup> in NW Europe, the latter supplying 0.7–0.75 of the nutritional needs of ruminants (Smit *et al.*, 2008). Although estimates are highly variable, all clearly indicate that grazed grass is the cheapest high quality feed available to ruminants and high quality conserved forage is cheaper than concentrates. The ratio cost of grazed herbage: conserved herbage: concentrate of approximately 1:2:4 is a guide to relative feed costs. Consequently the cost to produce one litre of milk and the proportion of grazed grass in the cows' diet across the major milk producing countries of the world are strongly inversely correlated (Dillon *et al.*, 2008). Nevertheless, even high quality grassland imposes limits on animal production. This is due, among other factors to an imbalance between digestible and true protein and net energy; a high fill value also limits the intake potential of grass and silage.

While grazed grass and grass silage can sustain adequate animal production for much of the beef production cycle, concentrates are usually required to at least finish steers in readiness for slaughter. For example in a trial in the Republic of Ireland, in the final three months of the production cycle, steers offered only high quality pasture produced the equivalent of 0.4 kg day<sup>-1</sup> carcass gain compared to 0.87 kg day<sup>-1</sup> when fed *ad libitum* concentrates. Further, the pasture-finished animals had not reached acceptable slaughter weight, irrespective of the breed of steer in the trial which ranged from slow to fast maturing genotype crosses (Keane and Moloney, 2010). These data are typical of finishing beef cattle in grass-based systems.

In the above example pasture was high quality intensively managed grass.

Even long term ('old') grassland with a low content of 'sown species' can produce yields as high as grassland with a high content of desired species, provided standard of management is high, although nutritive value is more variable and generally lower. Nevertheless silage from these swards, while generally having lower digestibility and energy content, can produce carcass gains as high as corresponding reseeds from a similar area of land (e.g. Keating and O'Kiely, 2000). However, when managing these swards as semi-natural grassland further nutritional limits are imposed. Although there are very few direct comparisons between milk production from high quality intensively managed swards and seminatural swards (representing extremes in herbage nutritive value and production) milk yield can be calculated from nutritional information. This requires knowledge about the animal's requirements and intake capability, nutritive value of the herbage, both grazed and ensiled, and optimum N and P balance to ensure maximum use efficiency so that environmental burden is minimised.

As account needs to be taken of N utilized by rumen micro-organisms, dietary N is considered in terms both of true dietary protein digested in the intestine and rumen degradable protein i.e. DVE and OEB, respectively, in the Dutch protein evaluation system (van Duinkerken *et al.*, 2011). OEB is the difference between the potential microbial protein synthesis based on available rumen degradable protein and on available rumen degradable energy. Hence a negative OEB indicates that relative to rumen degradable energy, the diet is deficient in rumen degradable protein, whereas if positive, it is in excess, and so losses as urinary-N and environmental burden will be increased (van Duinkerken *et al.*, 2005).

Table 1. Nutritional characteristics of intensively managed perennial ryegrass (PRG, *Lolium perenne* L.) and semi-natural grassland grazed or ensiled. Data from Netherlands and UK, Bruinenberg *et al.* (2006) and Frame (1991)

Sward	Use	Yield	g N	MJ NE	g DVE	g OEB	FV	OMD	g DVE MJ <sup>-1</sup> NE
		Mg DM ha <sup>-1</sup>		kg-	<sup>1</sup> DM				
PRG	Grazing	8.0	36.3	6.9	96.0	72.0	0.89	0.820	13.8
	Ensiling	11.0	27.7	6.1	65.0	60.0	1.02	0.750	10.6
Semi-	Grazing	6.1	20.9	5.2	68.2	-10.0	0.93	0.750	11.8
natural	Ensiling	8.4	15.9	4.6	46.2	-17.4	1.07	0.618	7.6
NTE /	D			1 1.		CDD		1 11	1

NE = net energy; DVE = True protein digested in the intestine; OEB = rumen degradable protein balance; FV = Fill value (Zom *et al.*, 2012); OMD = Organic matter digestibility (proportion of DM).

Table 2. Potential milk production per cow per year based on energy supply and grass only

Sward	Use	Potential* DM intake	NE intake	Potential* milk produc- tion	DVE/NE required	Balance	Potential* milk production
		kg day <sup>-1</sup>	MJ day <sup>-1</sup>	kg FPCM cow <sup>-1</sup> year <sup>-1</sup>	potential		kg FPCM ha <sup>-1</sup> year <sup>-1</sup>
PRG	Grazing	18.5	129	9267	11.8	+	9155
	Ensiling	16.2	99	6379	11.2	_	9931
Semi-	Grazing	17.7	93	5252	10.2	+	4141
natural	Ensiling	15.4	71	3178	9.1	-	3964

\*Potential is theoretical maximum for healthy HF cows with unlimited access to feed; FPCM is fat and protein corrected milk. Under practical but good management conditions milk production will be 7.5–17.5% lower.

Calculation of milk production potential (and minimum N environmental burden) includes the above terms plus fill capacity of the animal and fill value of the feed (Table 1). Milk production is primarily energy driven by NEI then DVE, followed, of course, by other essential dietary components. The optimum DVE/NEI ratio varies with production level. For example, a 5000 or 8000 kg FPCM producing cow requires on average 90 and 115 MJ NE day<sup>-1</sup>, respectively, with a DVE/NEI ratio of approximately 10.3 and 11.5, respectively, and a target OEB of 0. Feeding lower ratios will decrease milk production (DVE shortage), but increase the efficiency of protein utilisation. Feeding higher ratios will not affect milk production, but decrease the efficiency of protein utilization and increase emissions.

Grass-only diets from intensively managed grassland have potential to produce about twice the amount of milk that extensively managed grasslands produce (Table 2) but in terms of required DVE/NEl only silage from the extensive sward is outside the range of dairy cow requirement. However, only intensive grazed grass will meet energy requirements of the high yielding dairy cow (>8000 kg FPCM per year), but OEB is high causing environmental problems. Silage from intensively managed grass has potential to meet the requirements of a cow producing about 6000 kg although DVE is slightly deficient. Nevertheless, to meet a modern dairy cow's production target, or to minimise environmental burdens, additional feed is necessary (Table 3). Quality of the herbage determines whether protein or energy is needed.
Sward	Use	Potential (kg FPCM)	DVE*	OEB*	Supplementary feed needed?
PRG	Grazing	9267	+	+	Yes. NE to increase N efficiency and reduce environmental burden
	Ensiling	6379	_	+	Yes. DVE to use energy efficiently, exploit milk production potential and NE to in- crease N efficiency and reduce environmen- tal burden
Semi- natural	Grazing	5252	+	_	Yes. NE to increase N efficiency and reduce environmental burden and OEB to sus- tain rumen functioning
	Ensiling	3178	_	_	Yes. DVE to use energy efficiently, exploit milk production potential and OEB to sus- tain rumen functioning

Table 3. Extent to which protein requirements are met by type of sward and herbage use for potential milk yield based on energy supply

\* - = deficiency; + = excess; 0 = sufficient.

#### **Efficiency in use of inputs**

For ruminant production to be sustainable it should produce more human edible protein and energy than it consumes as an input. In the example in Table 4, the additional 4.4 kg FPCM were produced from 3.1 kg concentrates, with a slight decline in grass intake due to the substitution effect (Delagarde *et al.*, 2011).

Table 4. Potential daily feed intake and milk production of grazing cows in extensively managed grasslands receiving a range of concentrate levels

		% NE from concentrates									
	0	5	10	15	20	25					
Grass (kg DM)	17.7	17.4	17.0	16.7	16.3	15.9					
Concentrate (kg DM)	0.0	0.6	1.2	1.9	2.5	3.1					
Substitution (kg DM)	0.0	-0.4	-0.7	-1.1	-1.4	-1.8					
Total (kg DM)	17.7	18.0	18.3	18.5	18.8	19.1					
NE intake (MJ)	93.0	95.7	98.5	101.2	104.0	106.7					
Milk (kg FPCM)	14.4	15.3	16.2	17.1	17.9	18.8					

If the input energy had been derived from a human edible form of energy e.g. wheat grain, all other conditions being equal, the grain would more gainfully have been used as a direct source of feed. However, the additional milk also would have contained about 145 g protein. Assuming edible protein content of wheat to be 100 g kg<sup>-1</sup>, 310 g protein was used to produce 145 kg milk protein i.e. an inefficient use of edible protein. However, Wilkinson (2011) has calculated that only about 0.35 of compound feeds sold in the UK for milk production comprises human edible dry matter, by-products in concentrate formulation reducing reliance on human edible components. If this is applied *pro rata* for energy and protein, 0.35 of 310 g of protein is 109 g i.e. 109 g edible protein produces 145 g milk protein.

Wilkinson (2011) has compared the efficiency of systems of ruminant production (feed conversion ratios, FCR) to convert energy and protein into human edible forms when total and human edible energy and protein are considered as inputs in some UK animal production systems (Table 5). As an efficient system will produce more than it consumes, only in milk is more edible energy and protein produced than is consumed i.e. FCR is less than 1.

In some systems, the requirements of the mother need to be taken into account in calculating the input, especially concentrates, to producing the ruminant product (e.g. suckler cow in beef production and ewe in lamb production). So in some extensive systems, the FCR can be high. Nevertheless, the proportion of total energy and protein that is non-edible in the diet of ruminants is higher than in poultry and pig production.

	MJ total energy	kg total protein kg-1	MJ edible energy	kg edible pro-
Product	input MJ <sup>-1</sup> edible	edible protein pro-	input MJ <sup>-1</sup> edible	tein kg-1 edible
	energy produced	duced	energy produced	protein produced
Milk (6,500 litres)	4.5	5.6	0.47	0.71
Lowland suckler beef	37.0	23.8	4.2	2.0
Cereal beef	13.2	8.3	6.2	3.0
Upland suckler beef	40.0	26.3	1.9	0.92
Lowland lamb	52.6	30.3	2.5	1.1
Pig meat	9.3	4.3	6.3	2.6

Table 5. Feed conversion ratios (FCR) for total and human edible energy and protein as inputs to produce edible energy and protein in animal production systems (from Wilkinson, 2011)

Scope to improve the efficiency in the use of edible energy and protein includes increasing the contribution of high quality forages in ruminant diets and more widespread use of by-products. However, as more use is made of by-products in the food industry, this benefit may be less available and so more emphasis will be placed on high quality forage to achieve a low FCR (low edible input per unit of edible output).

#### Role of legumes in grassland sustainability

Some forage legumes such as red clover (Trifolium pratense L.) and lucerne (Medicago sativa L.), produce high DM yields and the silages they produce can have high protein content (g kg<sup>-1</sup> DM) e.g. 200 for red clover, 230 for lucerne (Marley et al., 2003). Potentially legumes confer benefits on animal production systems relative to grass swards receiving N fertilizer due to a) their ability to 'fix' N biologically, hence replacing N fertilizer, b) increased potential DM intake, due to more rapid passage of ingested herbage and c) higher feed conversion (although highly variable) (Peyraud et al., 2009; Dewhurst et al., 2009). The potential of legumes in mixtures is expressed most fully in the absence of the grazing animal (e.g. Nyfeler et al., 2009). However, taking a grass/white clover (Trifolium repens L.) sward as an example, the scale of benefit is usually not fully realised in animal production systems (over a grazing season or an annual animal production cycle involving grazed and conserved herbage). The ceiling yield of grass/white clover is usually 20 to 30% less than that of grass receiving high rates of N fertilizer in these systems. Further, under well managed production systems in which herbage allowance, especially during grazing, is controlled, the advantage of the presence of the legume to intake potential is not fully expressed. For example, averaged over 15 systems-type experiments, milk and beef production from grass swards with a good white clover content (annual average of about at least 250 g kg<sup>-1</sup> DM) is about 75 to 80% of a grass sward receiving 300 kg N ha<sup>-1</sup> or more (Figure 1). While individual animal performance in the beef experiments is generally slightly higher in the legume than the pure grass sward individual cow performance is similar between the two sward types.

Regarding emissions from legume systems, as N fertilizer application is small or zero, fossil energy dependence in the life cycle of the system is reduced (and lower carbon dioxide emission), as are direct nitrous oxide emissions as fixed N emits much less than N



Figure 1. Milk ( $\blacksquare$ ) or beef ( $\blacktriangle$ ) production per ha for grass/clover swards relative to comparable swards receiving a range of rates of nitrogen fertilizer plotted against difference in nitrogen applied to grass/ clover and sward receiving higher rates (adapted from reviews by Humphreys *et al.*, 2009 and Davies and Hopkins, 1996)

fertilizer. However, higher P requirement of legumes and high content of rapidly degradable protein confer potentially higher P and urinary N emissions, respectively. Judicious management of P can avoid major losses of that element. In grass/clover systems, nitrate leaching and nitrous oxide emissions from excreta are generally similar to grass receiving N fertilizer when N input is similar (Ledgard et al., 2009). However, as grass/legume will have a higher N content than grass-plus-N, for a given availability of herbage DM, DVE will be higher in the former. This will usually require legume diets to be supplemented with more NE than a grass sward producing a similar amount of DM to reach DVE/OEB balance. The grass-legume-rhizobium (nitrogen fixing bacteria) system confers advantages in the early life of mixtures due to the grass benefitting from the transferred fixed N from the legume and the N fixation rate increasing due to reduction in soil mineral N content (Nyfeler et al., 2011). However, this is not an equilibrium as the grass, in time, becomes dominant over the legume and the system becomes a victim of a cycle of falling and rising legume content and contribution of fixed N. This results in high variability in herbage production and nutritive value. Hence mixed grass/legume swards are often perceived as being unpredictable and, with a few exceptions, prone to cause bloat. However, if the standard of grassland management is high these in extremes can be minimised and high dependence can be placed on grass/cover swards.

#### Grass-based systems and emissions

Nitrogen and phosphorus cycles in food production systems are not closed. Even when all the nutrients that are removed from the cycle are replaced by purchased inputs (feeds and



Figure 2. Estimates of efficiency of N transition at emission spots for De Marke Knowledge Transfer Centre in Dairyman, a project in the EU INTERRREG IVB program, (data marked \* and in italics) and for an average dairy farm in the Netherlands (data in boxes). Measures contributing to increased efficiency of N transmission are indicated for each emission spot fertilizers) they are not totally incorporated into the system and a proportion of the nutrients introduced is lost. These losses can become environmental burdens when they exceed the natural capacity of the food production system. Eliminating emissions completely is not possible but they can be minimised by increasing the efficiency of utilisation. Losses may take place whenever the mineral makes a transition. So these 'emission spots' need to be identified and the efficiency of transition at each spot has to be determined. Low efficiency equals high loss (emission). In Figure 2 a basic N cycle of a dairy farm is used as an example to show the emission spots in a nutrient cycle, efficiency of transition and type of measures adopted to maximise efficiency.

Comparison of efficiency in N transition between de Marke and the average national farm in the Netherlands emphasises the potential to reduce N emissions. By adopting the de Marke model overall nitrogen use efficiency in the Netherlands has potential to increase by 40% (from 26 to 37 percentage units). A combination of farm nutrient management planning, fine tuning manure and fertiliser application rates and timing, farmer training and appropriate legislation all contribute to maximising efficiency of N use. Nitrification inhibitors to reduce nitrous oxide and nitrate emissions (Dawson *et al.*, 2011) and breeding grasses with reduced proteolytic characteristics to reduce protein breakdown in the rumen (Kingston-Smith *et al.*, 2010) may prove useful.

The phosphorus cycle is in some ways simpler than the nitrogen cycle as there are no gaseous losses and so it is easier to account for the fate of the mineral. Other than knowledge of soil P status and P fertiliser and manure application rates, knowledge of P content of bought-in feeds is necessary to manage P effectively and to minimise P loss. Vulnerable transition points for phosphorus include application of P fertiliser when soil P status is high (Watson *et al.*, 2007) and when manure, especially slurry, is applied when overland flow events occur soon after (O'Rourke *et al.*, 2010). Concentrates in particular have high P content; however, it has been shown in the UK that recommended P content of concentrates for dairy cows can be reduced by 20% without harmful effects to animal performance, health or welfare (Ferris *et al.*, 2010). Consequently P content in manures can be reduced to alleviate the level of emission at manure spreading.

#### Methane

Methane accounts for 40–50% of GHG emissions (excluding C sequestration) in ruminant production systems. As it is primarily produced in the rumen as a result of enteric fermentation, diet is the first step in minimising its impact, but mitigation options involving biotechnologies and additives are being pursued (Martin *et al.*, 2009). Feeding maize silage has a beneficial effect in reducing both nitrogen and methane emissions (Luo *et al.*, 2008; Schils *et al.*, 2007). Increasing dietary fat also results in lower methane emission, more so in sheep than cattle (Grainger and Beauchemin, 2011).

As high fibre diets tend to produce more methane per unit of intake or digested forage, emission will be higher in permanent and semi-natural pastures than in young reseeded grassland. Shorter cycles in beef and lamb production and longer dairy cow life also reduce methane emissions per unit of production. In the long term, selection for animals for increased production efficiency per unit of GHG emitted is a strong prospect, as variation already exists within herds and flocks (Waghorn and Hegarty, 2011; Bell *et al.*, 2011). So the greatest potential for reduction per unit of animal product is from intensively managed grassland using animals of appropriate genetics, including high production potential. Of course, the trade off with other emissions needs to be taken into account, as does that of feeding human edible food to ruminants that is discussed earlier.

#### Nitrous oxide

Nitrous oxide is the most virulent of the greenhouse gases so despite the low quantity emitted it makes a high contribution to total direct GHG emissions. The main sources of nitrous oxide are from soils (depending on organic content), excreta from grazing animals, inorganic N fertilizer and manure application (IPCC, 2006). Mitigation includes increasing reliance on forage legumes and reducing the length of the grazing season (as less nitrous oxide is lost from a covered slurry store than from directly voided excreta). Increasing ingested N utilisation efficiency, discussed earlier, also makes a contribution to mitigation. This latter measure is only effective if the reduced content of N in manures is not replaced by fertilizer N which is a further source of nitrous oxide and also other emissions associated with its manufacture and contribution to whole farm N surplus. Nitrification inhibitors can be an effective means of reducing nitrous oxide emissions from N fertilizer or urine. In a meta analysis of 113 data sets from 35 studies, nitrous oxide emission was reduced by a mean of 38% when nitrate inhibitor was incorporated with N fertilizer, relative to the fertilizer on its own (Akiyama et al., 2010). The mean reduction in nitrous oxide emission from urine patches from 46 comparisons in New Zealand of urine patches treated with the nitrification inhibitor DCD at a minimum rate of 10 kg ha<sup>-1</sup> was 57% although the range was 0 - 86% (de Klein *et al.*, 2011). The feasibility of applying inhibitors, especially to grazed grassland, is still under study.

### Carbon sequestration

The capability of soils of European grasslands to sequester carbon is a potential mitigation of GHG emissions. European grassland soils store, on average, about 95 Mg C ha<sup>-1</sup> (Smith *et al.*, 2007). In a pan-European study, the average rate of sequestration in mown grassland was 2.6 Mg CO<sub>2</sub>e ha<sup>-1</sup> and in grazed grassland 4.8 Mg CO<sub>2</sub>e ha<sup>-1</sup> (Soussana *et al.*, 2010) although variance was high (at least 0.9 Mg CO<sub>2</sub>e ha<sup>-1</sup>). In grassland soils carbon tends to accumulate until an equilibrium is reached which depends on characteristics of the existing organic matter, soil texture, and climate (Johnston *et al.*, 2009) so not all soils will accumulate carbon. Management interacts with soil moisture causing year-to-year variation even under constant grazing conditions (Klumpp *et al.*, 2011). Nevertheless, C sequestration has potential to offset at least part of the GHG emissions, Schils *et al.* (2007) calculating that it offset as much as 40% of emissions in a dairy system in the Netherlands.

When renewing a sward, cultivation (possibly minimal) and reseeding are necessary. Although cultivation will reduce soil C content, the higher producing replacement may 'capture' more C than the previous sward. If a crop with high photosynthetic potential is sown annually, recurring accumulation of C in the vegetation, if not in the soil, may eventually offset soil C losses e.g. maize vs grass/maize rotation. (Vellinga and Hoving, 2011). This example demonstrates the need to a) take account of the whole ecosystem when estimating C sequestration in contrasting systems and b) calculate rates over succeeding years.

## **Carbon footprint**

Although C footprints and life cycle assessments (LCA) of grassland systems and ruminant products lack a standard approach, comparisons of systems, especially within a study, highlight the most important processes or stages in a production cycle that contribute to GHG emissions and hence identify potential opportunities for mitigation. Applying whole farm models to calculation of total GHG emissions helps also to identify possible trade-offs or pollutant swaps when applying a mitigation scenario for a specific emission. Examples range from 4.22 Mg CO<sub>2</sub>e ha<sup>-1</sup> (including 2.79 Mg CO<sub>2</sub>e ha<sup>-1</sup> from nitrous oxide emissions from

organic soils but excluding C sequestration) for a hill farm in Wales, UK (Edwards-Jones *et al.*, 2009) to 21.0 Mg  $CO_2e$  ha<sup>-1</sup> for GHG emissions on a dairy farm practicing extended grazing in the UK and modelled to have a high N surplus (Schils *et al.*, 2007). In fact, this latter case exemplifies the relationship between N surplus and GHG emissions on a dairy farm and how introducing N surplus reduction strategies resulted in unintentional, but synergistic, reduction in GHG emissions.

Direct emissions (i.e. those originating directly from the farm and not from inputs or off-farm processes) have been estimated to accounted for 0.78 to 0.83 total emissions in Irish beef production. In a beef/sheep production system in upland Wales they accounted for over 0.90 of total emissions (Edwards-Jones et al., 2009). They account for 0.73 of total emissions for the average Dutch dairy farm so, generally, the more intensive the system of production, the smaller is the contribution of direct emissions to the total GHG budget. Some extensive systems are on high organic matter soils, which emit high amounts of nitrous oxide. This coupled with low stocking rates, conforming to agri-environmental guidelines, exacerbates the high carbon footprint per unit of animal production. There is no direct relationship between contribution of grassland to feed and low C footprint in beef production. Bull beef with a high input of concentrates and short lifecycle has a lower C footprint than beef from steers fed a high forage/low concentrate diet (Dawson et al., 2011; Foley et al., 2011). In a comparison between low and high forage systems and average and high milk fat and protein genotypes, the latter genotype in the low forage system had the lowest emissions (1.1 kg CO<sub>2</sub>e kg<sup>-1</sup> milk), when determined by partial LCA (Bell et al., 2011). When national C footprints are calculated, they are often much higher than those of the research centre farm or theoretical calculation, emphasising the relative inefficiency of the average farm. The difference also indicates the progress that can be made to improve adoption of mitigation measures on the average farm. Total emissions per kg carcass for a sample of specialised Irish sucker calf to beef were calculated to be 17% higher (23.1 vs. 19.7 kg CO<sub>2</sub>e kg<sup>-1</sup>carcass) than for a moderately intensive steer production system based on research

farm data (Foley *et al.*, 2011). Higher stocking rate and production efficiency were identified as the main factors in the lower emissions per kg carcass from the research farm scenario.

#### Animal production and biodiversity

Eighty one percent of Europe's permanent grassland and meadows and 44% of its total livestock units are in less favoured areas (LFAs) that include the most threatened grassland ecosystems due to abandonment as stock numbers decline.

Grazing management objectives differ between commercial production and conservation. However, high nature value grassland is often part of commercially farmed grassland and its management is dependent on commercial farmers. So conservation management prescriptions need to take this into account while not compromising biodiversity objectives. It is important that grazing management produces the necessary structural heterogeneity within the vegetation to encourage and support faunal and floral biodiversity and that can only be achieved by adopting stocking densities and grazing periods that would not be practical or desirable in commercial farming. In a trial extending over four European countries, ruminant liveweight gain from swards stocked to encourage biodiversity was on average about two thirds that of swards stocked at a rate acceptable for commercial animal production (Isselstein *et al.*, 2007). Further, it has been demonstrated above that semi-natural grassland with input of concentrates can achieve commercially feasible levels of milk production. So opportunities to integrate high biodiversity grassland into commercially productive grassland should be sought. Traditional breeds may be important for conservation management of hill land vegetation communities e.g. beef cattle on Scottish hills (Umstatter *et al.*, 2009), and the meat from such breeds may command a premium.

### Conclusions

While grazed grass is undoubtedly the cheapest source of high quality nutrients for ruminants, grassland-based ruminant production systems devoid of inputs are not sustainable. Nevertheless the consequence of the use of inputs needs always to be considered including the response, impact on environment or alternative use. Although some emerging technologies have been identified as potentially useful to improve the environmental footprint of grassland production systems, much can be done to improve management standards, particularly in reducing nutrient losses by applying existing techniques. This also applies to improving mitigation of emissions on a country scale where calculation of efficiency in use of inputs and C footprints of national ruminant production can lag behind calculations for research centres.

Some obvious trade-offs have been raised when mitigation strategies have been discussed, as have some dilemmas concerning the sacrifice of human food or land to produce food to sustain ruminant production. Whether concerned with optimising animal production, reducing emissions or minimising C footprint of animal product, high quality grassland and forage have an important role to play.

### References

Akiyama H., Yan X.Y. and Yagi K. (2010) Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N(2)O and NO emissions from agricultural soils: meta-analysis. *Global Change Biology* 16, 1837–1846.

Bell M.J., Wall E., Russell G., Simm G. and Stott (2011) The effect of improving cow productivity, fertility, and longevity on the global warming potential of dairy systems. *Journal of Dairy Science* 94, 3663–3678.

Bruinenberg M.H., Geerts R.H.E.M., Struik P.C. and Valk H. (2006) Dairy cow performance on silage from semi-natural grassland. *Netherlands Journal of Agricultural Science* 54, 95–110.

Copa-Cogeca (2009) Livestock and climate change. Copa-Cogeca Climate Change Series.

Davies D.A. and Hopkins A. (1996) Production benefits of legumes in grassland. In: Younie D. (ed.) *Legumes in Sustainable Farming Systems*, British Grassland Society, Reading, UK, pp. 234–246.

Dawson L.E.R., O'Kiely P., Moloney A.P., Wylie A.R.G., Carson A.F. and Hyslop J. (2011) Grassland systems of red meat production: integration between biodiversity, plant nutrient utilisation, greenhouse gas emissions and meat nutritional quality. *Animal* 5, 1423–1441.

De Klein C.A.M., Cameron K.C., Di H.J., Rys G., Monaghan R.M. and Sherlock R.R. (2011) Repeated annual use of the nitrification inhibitor dicyandiamide (DCD) does not alter its effectiveness in reducing N2O emissions from cow urine. *Animal Feed Science and Technology* 166–167, 480–491.

Delagarde R., Valk H., Mayne C.S., Rook A.J., Gonzalez-Rodriguez A., Baratte C., Faverdin P. and Peyraud J.L. (2011) GrazeIn: a model of herbage intake and milk production for grazing dairy cows. 3. Simulations and external validation of the model. *Grass and Forage Science* 66, 61–77.

Dewhurst R.J., Delaby L., Moloney A., Boland T. and Lewis E. (2009) Nutritive value of forage legumes used for grazing and silage. *Irish Journal of Agricultural and Food Research* 48, 167–187.

Dillon P., Hennessy T., Shalloo L., Thorne F. and Horan B. (2008) Future outlook for the Irish dairy industry; a study of international competitiveness, international trade reform and requirement for change. *International Journal of Dairy Technology* 61, 16–29.

Edwards-Jones G., Plassmann K. and Harris I.M. (2009) Carbon footprinting of lamb and beef production systems: insights from an empirical analysis of farms in Wales, UK. *Journal of Agricultural Science* 147, 707–719.

Ferris C.P., Patterson C.P., McCoy M.A. and Kilpatrick D.J. (2010) Effect of offering dairy cows diets differing in phosphorus concentration over four successive lactations: 1. Food intake, milk production, tissue changes and blood metabolites. *Animal* 4, 545–559. Foley P.A., Crosson P., Lovett D.K., Boland T.M., O'Mara F.P. and Kenny D.A. (2011) Whole farm systems modelling of greenhouse gas emissions from pastoral suckler cow production. *Agriculture, Ecosystems and Environment* 142, 222–230.

Frame J. (1991) Herbage production and quality of a range of secondary grass species at five rates of fertilizer nitrogen application. *Grass and Forage Science* 46, 139–151.

Grainger C. and Beauchemin K.A. (2011) Can enteric methane emissions from ruminants be lowered without lowering their production? *Animal Feed Science and Technology* 166–167, 308–320.

Hemme T. (2010) International Farm Comparison Network Dairy Report 2010, Kiel, Germany.

Humphreys J., Casey I.A. and Laidlaw A.S. (2009) Comparison of milk production from clover-based and fertilizer N-based grassland on a clay-loam soil in a moist temperate climate. *Irish Journal of Agricultural and Food Research* 48, 189–207.

IPCC (2006) Guidelines for National Greenhouse Gas Inventories, IGES, Japan.

Isselstein J., Griffith B.A., Pradel P. and Venerus S. (2007) Effects of livestock breed and grazing intensity on biodiversity and production in grazing systems. 1. Nutritive value of herbage and livestock performance. *Grass and Forage Science* 62, 145–158.

Johnston A.E., Poulton P.R. and Coleman K. (2009) Soil organic matter: its importance in sustainable agriculture and carbon dioxide fluxes. *Advances in Agronomy* 101, 1–57.

Keane M.G. and Moloney A.P. (2010) Comparison of pasture and concentrate finishing of Holstein Friesian, Aberdeen Angus x Holstein Friesian and Belgian Blue x Holstein Friesian steers. *Irish Journal of Agricultural and Food Research* 49, 11–26.

Keating T. and O'Kiely P. (2000) Comparison of old permanent grassland, *Lolium perenne* and *Lolium multiflorum* swards grown for silage. 1. Effects of beef production per hectare. *Irish Journal of Agricultural and Food Research* 39, 1–24.

Kingston-Smith A., Edwards J.E., Huws S.A., Kim E.J. and Abberton M. (2010) Plant-based strategies towards minimising 'livestock's long shadow'. *Proceedings of the Nutrition Society* 69, 613–620.

Klumpp K., Tallec T., Guix N. and Soussana J.F. (2011) Long term impacts of agricultural practices and climatic variability on carbon storage in permanent pasture. *Global Change Biology* 17, 3534–3545.

Ledgard S., Schils R., Eriksen J. and Luo J. (2009) Environmental impcts fo grazed clover/grass pastures. *Irish Journal of Agricultural and Food Research* 48, 209–226.

Luo J., Ledgard S.F., De Klein C.E.M., Lindsey S.B. and Kear M. (2008) Effects of dairy farming intensification on nitrous oxide emissions. *Plant and Soil* 309, 227–237.

Marley C.L., Fychan R., Fraser M.D., Winters A. and Jones R. (2003) Effect of sowing ratio and stage of maturity at harvest on yield, persistency and chemical composition of fresh and ensiled red clover/lucerne bi-crops. *Grass and Forage Science* 58, 397–406.

Martin C., Morgavi D.P. and Doreau M. (2009) Methane mitigation in ruminants: from microbe to the farm scale. *Animal* 4, 351–365.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Lüscher A. (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology* 46, 683–691.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E. and Lüscher A. (2011) Grass–legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems and Environment* 140, 155–163.

O'Rourke S.M., Foy R.H., Watson C.J., Ferris C.P. and Gordon A. (2010) Effect of varying the phosphorus content of dairy cow diets on losses of phosphorus in overland flow following surface application of manure. *Journal of Environmental Quality* 39, 2138–2146.

Peyraud J.L., Le Gall A. and Lüscher A. (2009) Potential food production from legume-based systems in Europe: an overview. *Irish Journal of Agricultural and Food Research* 48, 115–135.

Schils R.L.M., Olesen J.E., del Prado A. and Soussana J.F. (2007) A review of farm level modelling approaches for mitigating greenhouse gas emissions from ruminant livestock systems. *Livestock Science* 112, 240–251. Smit H.J., Metzger M.J. and Ewert F. (2008). Spatial distribution of grassland productivity and lans use in Europe. *Agricultural Systems* 98, 208–219.

Smith J., Smith, P., Wattenbach M., Zaehle S., Hiederer R., Jones R.J.A., Montanarella L., Rounsevell M.D.A., Reginster I. and Ewert F. (2005) Projected changes in mineral soil carbon of European croplands and grasslands, 1990–2080. *Global Change Biology* 11, 2141–2152.

Soussana J.F., Tallec T. and Blanfort V. (2010) Mitigating the greenhouse gas balance of ruminant production systems through carbon sequestration in grasslands. *Animal* 4, 334–350.

Umstatter C., Waterhouse T and Holland J.P. (2009) Cattle foraging behaviour in Scottish hills-one more step towards sustainability. *Proceedings of the 9<sup>th</sup> Conference: Construction, Engineering and Environment in Livestock Farming*, Berlin, Germany, pp. 333–338.

Van Duinkerken G., Andre G, Smits M.C.J., Monteny G.J. and Šebek L.B.J. (2005) Effect of rumendegradable protein balance and forage type on bulk milk urea concentration and emission of ammonia from dairy cow houses. *Journal of Dairy Science* 88, 1099–1112.

Van Duinkerken G., Blok M.C., Bannink A., Cone J.W., Dijkstra J., Van Vuuren A.M. and Tamminga S. (2011) Update of the Dutch protein evaluation system for ruminants: the DVE/OEB<sub>2010</sub> system. *Journal of Agricultural Science* 149, 351–367.

Vellinga T.V. and Hoving I.E. (2011) Maize silage for dairy cows: mitigation of methane emissions can be offset by land use change. *Nutrient Cycling in Agroecosystems* 89, 413–426.

Waghorn G. and Hegarty R.S. (2011) Lowering ruminant methane emissions through improved feed conversion efficiency. *Animal Feed Science and Technology* 166–167, 291–301.

Watson C.J., Smith R.V. and Matthews D.I. (2007) Increase in phosphorus losses from grassland in response to Olsen-P accumulation. *Journal of Environmental Quality* 36, 1452–1460.

Wilkinson J.M. (2011) Redefining efficiency of feed use by livestock. Animal 5, 1014-1022.

Zom R.L.G., André G. and van Vuuren A.M. (2012) Development of a model for the prediction of feed intake by dairy cows: 1. Prediction of feed intake. *Livestock Science* 143 43–57.

## Session 1.1.

## Multi-species sward in grassland-based ruminant production systems

# Tall fescue and Italian ryegrass – an ideal mixture for intensive cutting management

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## Abstract

We established a trial in 2009 to compare the agronomic performance of tall fescue (*Festuca arundinacea*, Fa) with Italian ryegrass (*Lolium multiflorum*, Lm) growing in monoculture and in different mixtures of both species. Mixtures differed in the ploidy of the Italian ryegrass component (diploid vs. tetraploid) and the contribution of both species to the mixtures (1/4 or 1/8 of Lm seeds on a number base). Five cuts were taken in both 2010 and 2011. N-fertilization was around 300 kg N ha<sup>-1</sup> per year. Dry matter (DM) content and botanical composition and feeding value were determined at each cut. Lm outyielded Fa in the first year after sowing but Fa outyielded Lm in the second year. In one of the mixtures, transgressive overyielding occurred in both years. The proportion of Fa in the DM yield of the mixtures was initially much lower than the sown proportion but increased gradually. We conclude that mixtures of Fa and Lm are particularly suited for an intensive cutting management.

Keywords: tall fescue, Italian ryegrass, mixture, botanical composition, yield, digestibility

## Introduction

Dairy production in western Europe is evolving toward less grazing and more cutting of grassland. Italian ryegrass (Lm; *Lolium multiflorum* L.), tall fescue (Fa; *Festuca arundinacea* Schreb.) and *Festulolium* are the species that are traditionally recommended for cutting-only management. Italian ryegrass establishes very fast and has a pronounced production in the spring, producing highly nutritious and easily digestible forage. The main disadvantage of Italian ryegrass is its low persistence. Tall fescue, on the other hand, establishes slowly after sowing but it is very high yielding and persistent once established and resists well to abiotic stress. Although new, soft-leaved varieties have been developed, the main disadvantage of tall fescue remains its lower palatability and digestibility compared to Italian ryegrass. Since the 1970s, breeders have tried to combine the advantages of both *Festuca* and *Lolium* in one species by creating Festulolium (Thomas *et al.*, 2003). Species mixtures are another approach to exploit the advantages of different species (Nyfeler *et al.*, 2009). We compared the yield of monocultures of Italian ryegrass and tall fescue with mixtures of both species. Our hypothesis was that the mixture would overyield the monocultures owing to the different growing patterns of both species.

## Materials and methods

Tall fescue (Fa; cv. Castagne), diploid Italian ryegrass (Lm2; cv. Melclips), tetraploid Italian ryegrass (Lm4; cv. Melquatro) and four mixtures of Fa and Lm2 or Lm4 were sown in September 2009 on a sandy loam soil in Merelbeke, Belgium. In the mixtures, 1/4 or 1/8 of the tall fescue seeds were substituted by Lm2 or Lm4 seeds. Throughout the text, mixtures will be named by the ryegrass component in the mixture (1/4Lm2, 1/4Lm4, 1/8Lm4). Sowing density was 1000 viable seeds m<sup>-2</sup>, plot size was 7.8 m<sup>2</sup>. The trial was

a randomized complete block with three replicates. Dicot weeds were removed using the appropriate herbicide treatments in spring. Annual fertilization amounted to 300 kg N ha<sup>-1</sup>, 9 kg P ha<sup>-1</sup> and 360 kg K ha<sup>-1</sup>, divided in 5 fractions. Five cuts were harvested per year with a Haldrup plot harvester. Sub samples were taken for separation into the constituent species and for chemical analyses. Digestibility and N content were determined in 2010 using NIRS.

## **Results and discussion**

Due to the slow establishment of Fa, its DM yield in 2010 was 20% lower than that of Lm2. Transgressive overyielding (which means that the mixture is more productive than the most productive component in monoculture) occurred for the mixture 1/8Lm4 : 3.3% higher DM yield and 7% higher N yield than Lm2. The organic matter digestibility (OMD) of Fa was significantly lower than that of Lm; the digestibility of the mixtures was intermediate. The crude protein (CP) and dry matter (DM) contents of Fa were significantly higher than those of Lm; CP and DM content of the mixtures were again intermediate (Table 1).

In 2011 Fa overyielded Lm2 by 6%, and Fa in turn was overyielded by the mixture 1/8Lm4. DM content was again significantly higher for Fa compared to Lm. Fa content in the harvested DM in the mixtures was always lower than the sown proportion and was generally higher in combination with diploid Lm compared to tetraploid Lm (Table 1). Within a season, the Fa content was low during the first three cuts (April, May, June), and increased sharply during the last two cuts (July, September) (Figure 1a). This is in accordance with the growing pattern of Italian ryegrass: high spring growth, low summer growth. Fa content increased in the second year, which could be explained either by the decreasing persistence of Lm or by the yield of Fa that is reaching its full potential.

The non-significant transgressive overyielding that occurred in the mixture 1/8Lm4 can be explained by its more constant production through the season relative to the monocultures. In 2010, 1/4Lm4 reached 86% of the yield of pure Lm4 in the first cut, and 80% of the yield of Fa in the last cut. In 2011 the yield of this mixture was 92% of the yield of Lm4 in the first cut and 94% of the yield of Fa in the last cut (Figure 1b). The mixtures combine both the good spring growth and fast establishment of Lm and the good summer and autumn yield of Fa. The reason why transgressive overyielding only occurred with the mixture 1/8Lm4 is not clear.

column ar	e not signi	ficantly di	ifferent (1	ukey test,	P < 0.05				
			20	2011					
	DMY	DMC	OMD	СР	NY	FaC	DMY	DMC	FaC
	kg ha⁻¹	%	%	%	kg ha⁻¹	%	kg ha⁻¹	%	%
1/4Lm2	17317 <sup>a</sup>	18.5 <sup>abc</sup>	74.9 <sup>ab</sup>	12.1 <sup>b</sup>	336.3ª	16.7 <sup>b</sup>	16238ª	19.8 <sup>ab</sup>	36.9 <sup>b</sup>
1/4Lm4	17422ª	17.7 <sup>abc</sup>	75.4 <sup>ab</sup>	11.9 <sup>b</sup>	331.7ª	11.1 <sup>b</sup>	16985ª	19.2 <sup>b</sup>	29.5°
1/8Lm2	17082ª	19.2 <sup>ab</sup>	74.4 <sup>ab</sup>	12.0 <sup>b</sup>	328.4ª	26.2ª	16626ª	21.3 <sup>ab</sup>	55.4ª
1/8Lm4	17572ª	17.9 <sup>abc</sup>	74.8 <sup>ab</sup>	12.4 <sup>ab</sup>	347.7ª	14.9 <sup>b</sup>	17926ª	20.8 <sup>ab</sup>	39.2 <sup>b</sup>
Fa	14000 <sup>b</sup>	19.9ª	73.8 <sup>b</sup>	13.4ª	299.0ª	100.0	17476 <sup>a</sup>	22.0ª	100.0
Lm2	17009ª	17.3 <sup>bc</sup>	76.2ª	11.9 <sup>b</sup>	325.0ª	0.0	17354ª	20.5 <sup>ab</sup>	0.0
Lm4	16942ª	16.4°	76.5ª	11.8 <sup>b</sup>	320.1ª	0.0	16915ª	18.5 <sup>b</sup>	0.0

Table 1. Mean annual dry matter yield (DMY), dry matter content (DMC), organic matter digestibility (OMD), crude protein content (CP), nitrogen yield (NY) and tall fescue content (FaC) of monocultures and mixtures of tall fescue (Fa) and Italian ryegrass (Lm). Treatments with the same letter in the same column are not significantly different (Tukey test, P < 0.05)

Based on the results of this trial, we suggest that to overcome the slow early establishment of a newly sown tall fescue sward, a mixture of tall fescue and approximately 10% Italian ryegrass may be a good alternative. The yield of the mixture is expected to be close to



Figure 1. (a) Tall fescue (Fa) content in the DM yield of 4 mixtures of tall fescue and diploid Italian ryegrass (Lm2) or tetraploid Italian ryegrass (Lm4) over 10 consecutive cuts in 2 years. (b) Dry matter yield of tall fescue (Fa), tetraploid Italian ryegrass (Lm4) and a mixture of both species over 10 consecutive cuts in 2 years

that of a pure Italian ryegrass sward in the first cuts. Gradually the Italian ryegrass disappears and the tall fescue takes over.

#### Conclusions

A mixture of tall fescue, with 1/8 of tetraploid Italian ryegrass, tended to outyield the best monoculture in two successive years, and compensated for the weaknesses of both constituent species. Italian ryegrass is gradually disappearing form the mixtures whilst the importance of tall fescue is increasing.

#### References

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Lüscher A. (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology* 46, 683–691.

Thomas H.M., Morgan W.G. and Humphreys M.W. (2003) Designing grasses with a future – combining the attributes of Lolium and Festuca. *Euphytica* 133, 19–26.

## Four-species grass-clover mixtures demonstrate transgessive overyielding and weed suppression in a 3-year continental-scale experiment

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## Abstract

The use of multi-species mixtures has been proposed as one strategy to improve agricultural sustainability. We conducted a co-ordinated continental-scale field experiment across 31 sites to investigate the relative resource utilisation (measured as biomass yield) of monocultures and four-species mixtures. Overall, comparisons of the yield of sown species (weeds excluded) demonstrated strong yield benefits associated with mixtures. Over a threeyear duration, transgressive overyielding occurred in 79% of mixtures and at about 70% of sites, with an average yield ratio of 1.18 (mixture yield/best monoculture yield). Mixture yield was better than average monoculture yield in 99.7% of mixture communities. By year 3, mixtures contained 8% of weeds and, thus, were much more resistant to weed invasion than monocultures (43% weeds). The yield benefits were robust to changes in the relative abundance of the four component species. These benefits established quickly (within the first year), persisted for at least three years, and were evident across sites with very different soils and climatic conditions.

Keywords: mixtures, diversity effect, symbiotic N, fixation, yield, transgressive overyielding

## Introduction

Ecological research in relatively species-rich and nutrient-poor systems shows that reductions in plant diversity of randomly assembled communities reduce the yield of aboveground biomass (Cardinale *et al.*, 2007). If this was also valid under nutrient-rich, intensively managed conditions, then increased crop diversity in species-poor agronomic systems could improve the provision of ecosystem services. However, the paucity of multi-species agronomic experiments with more than two species that have been conducted across multiple sites, years and mixtures, means that general predictions about the benefits of multi-species mixtures remain largely untested. In addition, studies on diversity and ecosystem functioning have generally used experimental designs that ignore or confound important components of diversity (relative abundance, richness and identity of species).

## Materials and methods

At each of 31 sites, fifteen grassland communities (four monocultures and eleven fourspecies mixtures selected from two grasses and two legumes) were sown at two levels of sown abundance of seed, resulting in 30 experimental plots per site. Functional groups of plant species (legume/grass, fast/slow speed of establishment) were chosen to maximise beneficial interspecific interactions; legumes enhance symbiotic fixation of atmospheric nitrogen (Nyfeler *et al.*, 2009, 2011), and slow/fast combinations were intended to maximise sward cover by species with known different temporal patterns of development. The eleven mixture communities had systematically varying proportions according to a simplex design (Kirwan *et al.*, 2007): four mixtures dominated in turn by each species (sown proportions of 70:10:10:10 of each other species), six mixtures dominated in turn by pairs of species (40:40:10:10) and the centroid community (25% of each species). Species proportions at sowing were based on proportions of seed mass considered appropriate for monocultures for each species at a site. Plots were harvested by mowing, and forage subsamples were sorted into the component sown and weed species. Not all sites had data for all three years: sites with three years, n = 24; two years, n = 6; one year, n = 1. Within each site, we compared the annual dry matter yield of mixtures (Yield<sub>MIX</sub>) in the first full season after sowing to i) that of the average monoculture performance (Yield<sub>MEANMONO</sub>) and ii) that of the monoculture that performed best across all years (transgressive overyielding) (Yield<sub>MAXMONO</sub>). Transgressive overyielding was tested with a permutation test (Kirwan *et al.*, 2007).

#### **Results and discussion**

Here, we report the yield of sown species, which reflects the relative ability of the monocultures and mixtures to utilise available resources for the production of the intended species only. Sites differed considerably in their site productivity, and average yields ranged from about 18 t ha<sup>-1</sup> year<sup>-1</sup> to about 3 t ha<sup>-1</sup> year<sup>-1</sup>, demonstrating a very wide range of growth conditions and yield potential across the 31 sites in our continental-scale experiment. Across all sites, mixture yield exceeded Yield<sub>MEANMONO</sub> (overyielding) in 340 of 341 mixture communities (99.7%), with an average ratio of mixture to monoculture yield of 1.77. Comparing mixture yield against Yield<sub>MAXMONO</sub> across all available years at a site, transgressive overyielding occurred in 270 of 341 mixtures (79%) and was significant at 22 of 31 sites (~70%), with an average yield ratio of 1.18 (Figure 1). Across all sites, monocultures displayed much higher levels of weed invasion than mixtures. The average percentage of weed biomass in the total yield of monocultures increased over time (25% in year 1, 33% in year 2 and 43% in year 3); in contrast, weed biomass in mixtures remained consistently low (7% in year 1, 6% in year 2 and 8% in year 3).



Figure 1. Ratio of yield of each mixture community (11 at each site) to yield of the best-performing monoculture (Yield<sub>MAXMONO</sub>) at each site based on yield of sown species (excludes weed biomass). Ratio values >1 indicate transgressive overyielding. Each point represents the biomass of a mixture community that has been averaged across the two seed density levels and years. Sites arranged in order of decreasing yield of Yield<sub>MAXMONO</sub>.

In a meta-analysis of biodiversity experiments that compared monocultures and mixtures, Cardinale et al. (2007) found significant transgressive overyielding in only 10 out of 83 experiments and, on average, this took about 5 years to become evident. In contrast, we found a very high incidence of transgressive overyielding. Only synergistic interactions can produce transgressive overyielding, and we attribute the yield benefits to the *a priori* design of the mixtures to include a combination of high-yielding species, legumes, and fast- and slow-establishing species, all of which were expected to contribute to synergistic interspecific interactions. Although legume proportions in the mixtures declined over time, there were still significant diversity effects in year 3; note also that we did not include any adaptive management techniques that could enhance the incidence of legumes in the sward. In comparisons of the yield of sown species, the greater susceptibility of monocultures to weed invasion is another mechanism by which mixtures can gain advantage over monocultures; however, comparisons based on total yield discount this specific effect but still produced similar results i.e. transgressive overyielding occurred in ~60% of sites (Finn et al., unpublished). Finally, note that the species associated with Yield<sub>MAXMONO</sub> at each site was selected retrospectively; this species may not necessarily have been the one chosen (or preferred) by farmers when sowing a monoculture.

### Conclusions

Over a three-year duration, transgressive overyielding occurred in about 70% of sites, and mixtures were much more resistant than monocultures to weed invasion. Yield benefits established quickly (within the first year), persisted for at least three years, were robust for different relative proportions of the four species, and were evident across sites with very different soils and climatic conditions.

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#### References

Cardinale B.J., Wrigh, J.P., Cadotte M.W., Carroll I.T., Hector A., Srivastava D.S., Loreau M. and Weis J.J. (2007) Impacts of plant diversity on biomass production increase through time because of species complementarity. *PNAS* 104, 18123–18128.

Kirwan L., Lüscher A., Sebastià M.T., Finn J.A., Collins R.P., Porqueddu C., Helgadóttir Á., Baadshaug O.H., Brophy C., Coran C., Dalmannsdóttir S., Delgado I., Elgersma A., Fothergill M., Frankow-Lindberg B.E., Goliński P., Grieu P., Gustavsson A.M., Höglind M., Huguenin-Elie O., Iliadis C., Jørgensen M., Kadžiulienė Ž., Karyotis T., Lunnan T., Malengier M., Maltoni S., Meyer V., Nyfeler D., Nykanen-Kurki P., Parente J., Smit H.J., Thumm U. and Connolly J. (2007) Evenness drives consistent diversity effects in an intensive grassland system across 28 European sites. *Journal of Ecology* 95, 530–539.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Lüscher A. (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology* 46, 683–691.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E. and Lüscher A. (2011) Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotiv and non-symbiotic sources. *Agriculture, Ecosystems and Environment* 140, 155–163.

## Symbiotic nitrogen fixation of grass-clover leys under organic and conventional cropping

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## Abstract

Symbiotic di-nitrogen  $(N_2)$  fixation of legumes is a greenhouse gas-neutral source of N for agriculture and is the most important external source of N in organic farming. In an experiment, in which organic and conventional cropping systems have been compared at two fertilizer input levels since 1978, we tested (i) if organically cropped clover has a higher yield and fixes more N<sub>2</sub> because of lower N fertilizer input than under conventional cropping, and (ii) if low phosphorus (P) and/or potassium (K) input limit clover growth and  $N_2$  fixation under organic cropping. To compare performance of N2 fixation, we determined the proportion of N derived from the atmosphere ( $P_{Ndfa}$ ; %; <sup>15</sup>N natural abundance method) in clover. To compare clover growth performance we determined the yield of Trifolium pratense and T. repens. The amount of Ndfa ( $A_{Ndfa}$ ; g m<sup>-2</sup>) reflects total sward N<sub>2</sub> fixation and depends on clover growth and its P<sub>Ndfa</sub>. P<sub>Ndfa</sub> was high for all cropping systems and fertilizer levels, indicating that symbiotic N, fixation process in clover plants was not limited in any of the treatments. A<sub>Ndfa</sub> was comparable in all the treatments except the unfertilized control, where it was decreased due to low yields of the total sward and its clover component. In the organic treatments decreased yield of total sward had no effect on A<sub>Ndfa</sub> because the proportion of clover was increased. These results demonstrate that both organically and conventionally cropped grass-clover leys fully profited from symbiotic N<sub>2</sub> fixation.

Keywords: <sup>15</sup>N, symbiotic N<sub>2</sub> fixation, yield, natural abundance, *Trifolium* 

## Introduction

Substitution of mineral fertiliser N by an improved exploitation of symbiotic  $N_2$  fixation is an important contribution to sustainable and resource-efficient agriculture. In organic cropping systems symbiotically fixed  $N_2$  is the most important external source of N.

In conventionally cropped swards, strong yield benefits were achieved by mixing grasses and clovers in a Pan-European experiment at 33 sites in 17 countries (Kirwan *et al.*, 2007; Lüscher *et al.*, 2008; Nyfeler *et al.*, 2009). The contribution of symbiotically fixed clover N to total sward N yield reached up to 30 g N m<sup>-2</sup> yr<sup>-1</sup> (Nyfeler *et al.*, 2011; Zanetti *et al.*, 1997). These high amounts of symbiotically fixed N<sub>2</sub> were only reached if clover achieved high biomass yield and gained a large proportion of its N from symbiosis (Nyfeler *et al.*, 2011). For organically cropped grass-clover leys, however, such studies are rare.

## Materials and methods

In the long-term DOK trial (Mäder *et al.*, 2002), in which organic and conventional cropping systems have been compared at two fertilizer input levels since 1978, we aimed to get insight into the yielding ability and performance of symbiotic  $N_2$  fixation of clover as affected by cropping systems and fertilizer input. We tested the two hypotheses: (i) clover cropped organically grows more vigorously and fixes more  $N_2$  because of lower N fertilizer input

than under conventional cropping, and (ii) low phosphorus (P) and/or potassium (K) input limit clover growth and N<sub>2</sub> fixation under organic cropping.

The concept, experimental design and management practices of the DOK trial were presented by Mäder *et al.* (2002). Since 1978, three cropping systems have been applied: two organic (bio-dynamic = DYN; bio-organic = ORG) and a conventional (receiving mineral fertilizer and farm yard manure = CON). In DYN, ORG and CON fertilizer input was varied at two levels: amounts and forms of nutrient input typical for the respective cropping system (level = 2) and half of this (level = 1). Additionally, two control treatments were included: MIN was a system receiving only mineral fertilizer (at level 2), and NON was non-fertilized. For details see Oberson *et al.* (2010). All treatments have been cultivated in four replicates in the field with the same seven-year crop rotation in a Latin square splitsplit-plot design. The leys contained the following sown species: *Trifolium repens*, *T. pratense*, *Lolium perenne*, *Dactylis glomerata*, *Festuca pratensis* and *Phleum pratense*. N derived from the atmosphere was determined with <sup>15</sup>N natural abundance (Unkovich *et al.*, 1994).

#### **Results and discussion**

Table 1. Effect of cropping system and fertilizer level on yield and clover proportion of a grassclover ley ( $2^{nd}$  year after sowing, 2007) and on symbiotic N<sub>2</sub> fixation of red clover.

Cropping Systems: DYN = bio-dynamic, ORG = bio-organic, CON = conventional; Fertilizer levels: 2 = typical for respective cropping system, 1 = half of level 2; Controls: MIN = mineral fertilizer only, NON = unfertilized;  $P_{Ndfa}$  = Proportion N derived from atmosphere,  $A_{Ndfa}$  = Amount Ndfa; ns = not significant, \* *P* < 0.1, \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001

treatment		total sward		red clover				
CropSust	yield	yield	clover	yield	P <sub>Ndfa</sub>	A <sub>Ndfa</sub>		
EartLaval	annual	1 <sup>st</sup> cut	1 <sup>st</sup> cut	1 <sup>st</sup> cut	1 <sup>st</sup> cut	1 <sup>st</sup> cut		
rentLevel	g DM m <sup>-2</sup>	g DM m <sup>-2</sup>	%	g DM m <sup>-2</sup>	%	g m <sup>-2</sup>		
DYN1	1021	383	57	176	88	4.7		
DYN2	1061	468	56	242	89	6.3		
ORG1	983	387	60	178	90	5.0		
ORG2	1163	499	62	292	91	7.5		
CON1	1245	489	45	178	92	5.0		
CON2	1322	544	32	162	92	4.4		
NON	651	195	56	76	90	2.2		
MIN	1257	539	30	144	85	3.5		
SEM <sup>1</sup>	59	27	8.7	44	2	1.1		
CropSys <sup>2</sup>	**	**	*	ns	ns	ns		
FertLevel <sup>2</sup>	х	***	ns	ns	ns	ns		
CS x FL <sup>2</sup>	ns	ns	ns	ns	ns	ns		

<sup>1</sup>over all 8 treatments <sup>2</sup>for the three cropping systems DYN, ORG, CON and the two fertilizer levels 1 and 2.

Symbiotic N<sub>2</sub> fixation was the main source of N for both clover species examined. P<sub>Ndfa</sub> reached values between 85 and 92% for red clover (Table 1) and between 92 and 97% for white clover (data not shown). In both species P<sub>Ndfa</sub> was not affected by the cropping system or the fertilizer level and was high even in the unfertilized control. These high levels of P<sub>Ndfa</sub> are comparable to other studies with grass-clover leys under conventional cropping at moderate N fertilizer input (Zanetti *et al.*, 1998; Nyfeler *et al.*, 2011). Such high levels of P<sub>Ndfa</sub> indicate that growth conditions did not directly limit the process of symbiotic N<sub>2</sub> fixation (Lüscher *et al.*, 2011). Variation of mineral N input among the treatments from 0–12.2 g m<sup>-2</sup> yr<sup>-1</sup> (NON and MIN respectively) did not affect P<sub>Ndfa</sub> which is in accordance

with Nyfeler *et al.* (2011) who compared 5.0 and 15.0 g N m<sup>-2</sup> yr<sup>-1</sup>. Also the strong variation of inputs of P (0–4.1 g P m<sup>-2</sup> yr<sup>-1</sup>) and K (0–25.8 g K m<sup>-2</sup> yr<sup>-1</sup>) did not affect the process of symbiotic N<sub>2</sub> fixation *per se*. In all treatments, clover plants fully profited from symbiotic N<sub>2</sub> fixation to close the gap between availability of mineral N in the soil and their requirements of N for growth (Lüscher *et al.*, 2011).

Biomass yield (annual and 1<sup>st</sup> cut) of the total sward was decreased in the organic systems and the low fertilizer level (Table 1). This cropping system effect is in agreement with the long-term results from the DOK trial (Mäder *et al.*, 2002). However, clover proportion in the sward was increased under organic cropping (Table 1). Competitive ability seemed to be positively affected by the amount and/or form of N fertilizer input in organic cropping. The two effects: reduced yield of total sward but increased clover proportion counterbalanced each other resulting in no net effect of the cropping system on clover yield and  $A_{Ndfa}$ . Only in the unfertilized control yield decrease was not compensated by the increase in clover proportion.

#### Conclusions

The proportion of N derived from the atmosphere was very high in all cropping systems and fertilizer levels including the unfertilized control. Thus, treatments did not limit the process of symbiotic  $N_2$  fixation in clover. Decreased total sward yield under organic cropping was counterbalanced by increased proportion of clover resulting in comparable clover yields and  $A_{Ndfa}$  in organic and conventional systems. In this long-term experiment, both organically and conventionally cropped grass-clover leys fully profited from symbiotic  $N_2$  fixation.

#### References

Kirwan L., Lüscher A., Sebastià M.T., Finn J.A., Collins R.P., Porqueddu C., Helgadóttir Á., Baadshaug O.H., Brophy C., Coran C., Dalmannsdóttir S., Delgado I., Elgersma A., Fothergill M., Frankow-Lindberg B.E., Golinski P., Grieu P., Gustavsson A.M., Höglind M., Huguenin-Elie O., Iliadis C., Jørgensen M., Kadžiulienė Ž., Karyotis T., Lunnan T., Malengier M., Maltoni S., Meyer V., Nyfeler D., Nykanen-Kurki P., Parente J., Smit H.J., Thumm U. and Connolly J. (2007) Evenness drives consistent diversity effects in an intensive grassland system across 28 European sites. *Journal of Ecology* 95, 530–539.

Lüscher A., Finn J.A., Connolly J., Sebastià M.T., Collins R., Fothergill M., Porqueddu C., Brophy C., Huguenin-Elie O., Kirwan L., Nyfeler D. and Helgadóttir Á. (2008) Benefits of sward diversity for agricultural grasslands. *Biodiversity* 9, 29–32.

Lüscher A., Soussana F. and Huguenin-Elie O. (2011) Role and impact of legumes in grasslands for high productivity and N gain from symbiotic N<sub>2</sub> fixation. In: Lemaire G., Hodgson J. and Chabbi A. (eds) *Grassland productivity and ecosystem services*, CAB International, Wallingford, UK, pp. 101–107.

Mäder P., Fliessbach A., Dubois D., Gunst L., Fried P. and Niggli U. (2002) Soil fertility and biodiversity in organic farming. *Science* 296, 1694–1697.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Lüscher A. (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology* 46, 683–691.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E. and Lüscher A. (2011) Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems and Environment* 140, 155–163.

Oberson A., Tagmann H.U., Langmeier M., Dubois D., Mäder P. and Frossard E. (2010) Fresh and residual phosphorus uptake by ryegrass from soils with different fertilization histories. *Plant and Soil* 334, 391–407. Unkovich M.J., Pate J.S., Sanford P. and Armstrong E.L. (1994) Potential precision of the d<sup>15</sup>N natural abundance method in the field estimates of nitrogen fixation by crop and pasture legumes in south-west Australia. *Australian Journal of Agricultural Research* 45, 119–132.

Zanetti S., Hartwig U.A., Van Kessel C., Lüscher A., Hebeisen T., Frehner M., Fischer B.U., Hendrey G.R., Blum H. and Nösberger, J. (1997) Does nitrogen nutrition restrict the CO<sub>2</sub> response of fertile grassland lacking legumes? *Oecologia* 112, 17–25.

# Exploring the relationship between diversity and productivity of a semi-natural permanent grassland using plant functional traits

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## Abstract

Recent studies have indicated that plant functional traits are important instruments for analysing functional diversity of grasslands. We present the results of a study on the relationship between diversity and productivity conducted in the Solling Uplands, Germany. The experimental site is an old permanent semi-natural grassland, with no history of arable farming. The three-factorial Latin square design with six blocked replications includes sward type (control, monocot-reduced and dicot-reduced by herbicide application), mowing frequency (1 and 3 cuts per year) and fertilization intensity (no fertilization and 180–30–100 kg ha<sup>-1</sup> of N-P-K) as main factors, and block and row effects as spatial factors. On each plot, we collected information on six plant functional traits for the main species making up 80% of the yield: plant height, green leaves/total leaves ratio, leaf dry matter content, stem dry matter content, stem specific density, and specific leaf area. We found that the functional diversity index (FD), defined as the total branch length of a functional dendrogram based on a species-traits matrix, increases with higher cutting frequency and decreases with higher fertilizer input. The main factor influencing the differences in the functional diversity index is the number of species making up 80% of the yield.

Keywords: functional diversity index, fertilization, cutting, management

## Introduction

Agricultural intensification in the twentieth century, driven by increasing needs for food production, resulted in changing grassland management practices in Europe by increasing both mowing frequency and nutrient input, and turning natural grasslands to monocultural stands. Hooper *et al.* (2005) report on several biodiversity-related experiments that have been established in order to investigate the relationship between diversity and productivity of grasslands. Diaz and Cabido (2001) summarized results of the studies on plant functional diversity and found that plant functional diversity matters for ecosystem processes. In our study, we focused on the relationship between functional diversity and productivity of the seminatural grassland and the influence of management factors.

## Materials and methods

The study site, situated in the Solling uplands (60 km from Goettingen, Germany), was established in 2008 on a permanent semi-natural grassland. The current vegetation is a nutrient poor, moderately wet *Lolio-Cynosuretum* community. Three management factors were replicated six times in a Latin square design: sward diversity (Control, dicot-reduced (–Dic), monocot-reduced (–Mon)); fertilization (no, or 180–30–100 of N-P-K); and cutting frequency (cut once or three times per year). Two vegetation surveys were conducted in May and September 2010 after Klapp and Stählin (1936). According to the results of these surveys, we

selected the main species for the analysis that together made up about 80% (or more) of the yield on each of the  $15 \times 15$  m plots. In 2011, we took samples three times: in the middle of May, beginning of July and beginning of September. Plants were cut at ground level (2 individuals of each species per plot) and transported to the lab in plastic bags with a wet tissue inside. They were stored at 9°C in the dark prior to analysis. The following plant functional traits were selected due to their potential reaction to the management applied (Cornelissen et al., 2003; Duru et al., 2005): plant height, leaf dry matter content, stem specific density, total leaves/green leaves ratio, leaf specific area, stem dry matter content. Plant functional traits were measured according to the standardised protocol by Cornelissen et al. (2003) two weeks before each cutting. Fresh biomass was determined in the field at harvest (cutting height 7 cm, Haldrup<sup>®</sup> forage combine harvester). Dry biomass was calculated accounting for water content of sub-samples of 100–200 g after drying them at 60°C for 48 h. Statistical analysis of the data was performed in R (Version 2.13.1, R Development Core Team 2011). We calculated a functional diversity index (FD), defined as the total branch length of a functional dendrogram of a species-traits matrix, after Petchey and Gaston (2002), for each plot based on the six selected plant functional traits for dominant species making up 80% of the yield. Analysis of variance (ANOVA) was performed on linear models for yield and FD with block and row as environmental factors to take into account spatial variability of the experimental area and sward, fertilization and cutting frequency as fixed effects. We used Canoco for Windows version 4.5 (1997-2004) for multivariate statistical analysis of the vegetation surveys and applied log transformation to the species scores.

#### **Results and discussion**

Only up to 8.3% of the overall yield in 2011 was explained by the functional diversity index (P = 0.019). This percentage changed depending on the cutting event (data not shown). A reason for the low explanatory power of the index could be the climatic situation in July 2011: Due to a drought early in the season, other species dominated biomass production than in 2010. The average annual FD was similar for all three sward types. It was, however, larger in plots that were cut three times a year than in plots cut once a year and smaller in fertilized than in unfertilized plots (Table 1).

Table 1. Average functional diversity index (FD) according to the treatments, FD coefficient of variation and influence of the main experimental factors on the functional diversity index in the year 2011 (block and row included in the analysis of variance as spatial factors). –Dic stands for dicot-reduced sward type, –Mon stands for monocot-reduced sward type

Factors		Sward			zation	Cutting frequency	
Levels	Control	–Dic	-Mon	No	NPK	1x	3x
Average functional diversity	16.81	17.04	16.51	17.30	16.27	15.84	17.73
FD coefficient of variation, %	13.36	13.51	13.32	12.21	13.82	13.54	10.74
FD variance explained by the corresponding factor, %	0.97			5.47		14.98	
Effect $P > F$		0.61, ns		0.01*		0.001***	

FD varied significantly among blocks (14.98% variance explained, P = 0.014), but not among rows (4.33% variance explained, P = 0.5). This could be due to the fact that one of the most dominant species, *Festuca rubra*, was more abundant in plots with increasing block number. The positive relation between cutting frequency and FD values could be due to slower growing species receiving less light in fertilized plots so that they could not become dominant there. They would, however, have had better chances on plots that were cut three times a year.

According to Grime (1998), ecosystem functioning is mainly determined by the trait values of the dominant contributors to plant biomass. We tested the correlation between FD and species number, making up 80% of biomass production. 83% of the FD was explained by the number of species (P < 0.001). Increase in number of dominant species increased the FD (cor = 0.89, P < 0.001) and decreased the yield (cor = -0.312, P = 0.008). A partial PCA testing the significance of the main species' contribution to the FD (Principal components analysis ordination method) indicated that an increase of the FD was explained largely by the presence of the following species: *Veronica chamaedrys, Holcus mollis, Cerastium holosteoides, Cirsium arvense* and *Rumex acetosa* (according to the Monte Carlo permutation test all treatments explained significant shares of the vegetation variance, P < 0.05). Diaz *et al.* (2007) reported that functional diversity influences ecosystem properties and services. We suppose that although the correlation between overall yield and the functional diversity index was not too strong (-0.28, P = 0.018), it seems that having more dominant species compared to only few could be beneficial for ecosystem processes.

### Conclusions

Nutrient input reduced the functional diversity of the sward in comparison to unfertilized plots whereas intensification of use increased the FD. The main factor explaining the FD was the number of species making up 80% of the yield.

#### Acknowledgements

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#### References

Cornelissen J., Lavorel S., Garnier E., Diaz S., Buchmann N., Gurvich D., Reich P., ter Steege H., Morgan H., Van der Heijden M., Pausas J. and Poorter H. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* 51, 335–380.

Diaz S. and Cabido M. (2001) Vive la difference: plant functional diversity matters for ecosystem processes. *Trends in Ecology and Evolution* 16-11, 646–655.

Diaz S., Lavorel S., de Bello F., Quetier F., Grigulis K. and Robson M. (2007) Incorporating plant functional diversity effects in ecosystem service assessments. *Proceedings of the National Academy of Sciences* 104, 52. Duru M., Tallowin J. and Cruz P. (2005) Functional diversity in low-input grassland farming systems: characterisation, effect and management. *Agronomy Research* 3 (2), 125–138.

Grime J.P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology* 86, 902–910.

Hooper D., Chapin F., Ewel J., Hector A., Inchausti P., Lavorel S., Lawton J., Lodge D., Loreau M., Naeem S., Schmid B., Setälä H., Symstad A., Vandermeer J. and Wardle D. (2005) Effects of biodiversity on current ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75 (1), 3–35.

Klapp E. and Stählin A. (1936) Standorte, Pflanzengesellschaften und Leistung des Grünlandes. Stuttgart, Ulmer.

Petchey O. and Gaston K. (2002) Functional diversity (FD), species richness and community composition. *Ecology Letters* 5, 402–411.

## Improved persistence of red clover (*Trifolium pratense* L.) varieties in mixed swards

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## Abstract

Red clover (*Trifolium pratense* L.) is an increasingly important forage legume for sustainable grassland systems, producing high dry matter (DM) yields of quality forage; however, currently available varieties lack persistence particularly under grazing. The focus of the IBERS red clover breeding programme is on identifying the factors contributing to the poor persistence of red clover swards and applying this information to the selection of improved germplasm, leading to the development of new varieties with greater persistence. Twelve red clover varieties and selection lines were grown in mixed swards with hybrid ryegrass or a mixture of hybrid ryegrass and perennial ryegrass over three harvest years. Three harvests were taken in each year with a Haldrup forage harvester at a cutting height of 5 cm and total DM yields and DM yields of the red clover varieties were observed and, although the yield in year 3 was generally lower than in year 1, varieties differed in the extent of this decline. Year-3 yield decline was less in the new varieties than in the control varieties of Milvus and Merviot. The implication of these results for the future use of red clover in sustainable grassland systems is discussed.

Keywords: Trifolium pratense, yield, persistence, variety

## Introduction

Sustainable livestock production systems are increasingly reliant on the production of high quality forage that can be produced 'on farm'. Red clover (*Trifolium pratense* L.) is an increasingly important forage legume in such systems, producing high dry matter (DM) yields of good quality forage (Frame *et al.*, 1997). However, under a typical management of 3 conservation cuts per year and a late autumn grazing, red clover swards tend to persist for only 3 years, after which the DM yields decline. In common with other red clover breeding programmes (Taylor, 2008), the objective of the IBERS red clover breeding programme has been the identification of the factors contributing to the poor persistence of red clover within swards and applying this information to the selection of improved germplasm leading to the development of red clover varieties that combine high forage yields with greater persistence. In spaced plants, crown diameter is the morphological characteristic most associated with plant mortality (Ortega and Rhodes, 1996). This paper summarises the progress of this programme and includes results from field trials quantifying the yield and quality of these new varieties and selection lines in comparison with current commercially available varieties.

## Materials and methods

Field plots (5 m × 1 m) of 12 red clover varieties comprising control varieties and selection lines were sown at IBERS, Aberystwyth in summer 2008 on soil of the Rheidol series. The red clover varieties were sown at a seed rate of 7.5 kg ha<sup>-1</sup> in mixed plots with hybrid ryegrass (*Lolium boucheanum* Kunth) cv. AberEcho sown at 35 kg ha<sup>-1</sup>, or with a mixture of perennial ryegrass (*Lolium perenne* L.) cv. AberDart sown at 12.6 kg ha<sup>-1</sup> and hybrid ryegrass cv. AberEcho sown at 22.4 kg ha<sup>-1</sup>. All plots were harvested three times in each of the following three harvest years with a Haldrup forage harvester at a cutting height of 5 cm. Fresh weights were measured and grass and red clover content analysed on a 300 g subsample from each plot. Dry matter yields of each were calculated after drying the subsample in a forced-draught oven at 80°C for 24 hours. The experiment was a split plot design with three replicate blocks with grass mixture as main plots and varieties as sub-plots. Total DM yield and red clover DM yield in each of the three harvest years were analysed by analysis of variance (ANOVA) using GenStat® Release 13 (Payne *et al.*, 2010) to discern significant effects of grass mixture and red clover variety.

#### **Results and discussion**

Increasing forage-based protein that can be grown 'on-farm' is one way of addressing the UK protein deficit and increasing the efficiency of livestock production. Red clover is a forage legume with a protein content of 18–19% crude protein (Frame et al., 1997) that can be grown across the UK. A major objective of the red clover breeding programme in Aberystwyth is to develop varieties with improved persistence and yield. The red clover varieties in the experiment differed significantly in DM yield (Table 1) in each of the three harvest years but, as there was no significant effect of companion grass, only the DM yield of the red clover fraction and the total plot DM yield are presented. In this experiment, the DM yield of the red clover varieties ranged from 7.5 to 22.6 t ha<sup>-1</sup> in year 1, from 9.4 to 22.6 t ha<sup>-1</sup> in year 2 and from 6.2 t ha<sup>-1</sup> to 16.0 t ha<sup>-1</sup> in year 3, while the highest total DM yield in each harvest year was 27.4 t ha<sup>-1</sup> in year 1, 24.1 t ha<sup>-1</sup> in year 2 and 20.7 t ha<sup>-1</sup> in year 3. The ranking of varieties reflected their performance over the 3 years and emphasised the difference in variety performance and their suitability for the UK environment. Although the variety Milvus produced the greatest red clover DM yield in year 1 and 2, the total DM yield and DM yield of the red clover fraction declined significantly in the third harvest year. In comparison, the DM yields of cv. AberClaret and Aa4559 remained relatively high in year 3, with the total DM yield and DM yield of the red clover fraction of both varieties 4 t ha<sup>-1</sup> more than Milvus and 6 t ha<sup>-1</sup> greater than Merviot.

V	Ye	ar 1	Ye	ar 2	Year 3		
variety	Red clover	Total	Red clover	Total	Red clover	Total	
Aa4557	16.2 (8)	21.5 (8)	17.7 (8)	20.2 (6)	9.7 (7)	15.9 (6)	
Aa4559	19.5 (5)	24.9 (3)	21.9 (2)	23.7 (2)	15.8 (2)	20.7 (1)	
Aa4560	14.2 (10)	19.1 (10)	13.3 (11)	15.6 (11)	6.3 (11)	13.4 (10)	
Aa4561	15.5 (9)	19.8 (9)	17.6 (9)	19.2 (9)	9.2 (8)	14.0 (= 8)	
Milvus	22.6 (1)	26.4 (2)	22.6 (1)	24.1 (1)	11.8 (5)	16.4 (5)	
AberChianti	19.8 (4)	24.3 (5)	21.5 (3)	22.8 (3)	13.9 (= 3)	17.8 (4)	
AberClaret	21.9 (2)	27.4 (1)	20.7 (5)	22.1 (5)	16.0 (1)	20.6 (2)	
Pavo	21.3 (3)	24.5 (4)	21.2 (4)	22.6 (4)	13.9 (= 3)	18.2 (3)	
Merviot	18.3 (6)	22.4 (7)	18.3 (7)	20.1 (= 7)	8.7 (9)	14.0 (= 8)	
Britta	14.1 (11)	18.5 (11)	15.3 (10)	17.4 (10)	7.2 (10)	12.9 (11)	
Vivi	7.5 (12)	13.6 (12)	9.4 (12)	11.4 (12)	6.2 (12)	11.4 (12)	
Amos	17.6 (7)	23.2 (6)	18.5 (6)	20.1 (= 7)	10.6 (6)	15.3 (7)	
s.e.d.	1.17	0.86	1.47	1.30	1.25	1.31	
Sign. *** $P < 0.001$	***	***	***	***	***	***	

Table 1. Red clover dry matter yield and total (red clover +grass) dry matter yield (t ha<sup>-1</sup>) of 12 red clover varieties and selection lines grown over 3 harvest years. Results are derived from a total of 3 cuts in each harvest year. (Rankings with columns shown in brackets)

Generally, the yields of all the varieties and selection lines within the experiment declined in the third harvest year compared with year 1 (Table 1). The varieties AberClaret, Aber-Chianti and the selection line Aa4559 have been selected for improved persistence and yield. Evidence from this experiment indicates that, although the yield of these varieties also declined in the third harvest year, the difference in yield between year 1 and year 3 was relatively small compared to the control varieties Milvus and Merviot. The decline in yield is a consequence of a loss in red clover plants per unit area, which is greater in some of the less-persistent varieties (data not shown). Increasing the duration of field trials beyond three harvest years will provide further evidence of the improved persistence of these varieties.

#### Conclusions

The development of red clover varieties with improved persistence is a target of the IBERS red clover breeding programme. Evidence from these field experiments over three harvest years shows that the new varieties maintain a high DM yield into the third harvest year and the yield decline between harvest years 1 and 3 is considerably less than in some of the commercially available varieties. Analysis of the DM yield of these new red clover varieties in the fourth and fifth harvest years will provide valuable information on their potential in sustainable grassland systems.

### References

Frame J., Charlton J.F.L. and Laidlaw A.S. (1997) Red clover. In: Frame J., Charlton J.F.L and Laidlaw A.S. (eds) *Temperate forage legumes*. CAB International, Wallingford, UK, pp.181–224.

Ortega F.M. Rhodes I. (1996) Characteristics associated with mortality in red clover (*Trifolium pratense* L.). In: Younie D. (eds) *Legumes in Sustainable Grassland Systems*. Occasional Symposium No. 30, British Grassland Society, pp. 92–93.

Payne R.W., Murray D.A., Harding S.A., Baird D.B. and Soutar D.M. (2010) Introduction to GenStat® for Windows<sup>TM</sup>. VSN International, Hemel Hempstead, UK.

Taylor N.L. (2008) A century of clover breeding development in the United States. Crop Science 48, 1–13.

## Effect of seed mixture composition and management on competitiveness of herbs in temporary grasslands

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## Abstract

In multispecies grasslands the proportion of different herb species may vary considerably due to low competitiveness of some herbs. To examine the possibility for increasing the competitiveness, an experiment with three factors was set up: 1) amount of herb seed (5, 50 or 100%) in a mixture of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), 2) cutting frequency, and 3) slurry application. The experiment was carried out over three years. The herb mixture contained salad burnet (*Sanguisorba minor*), fenugreek (*Trigonella foenum-graecum*), chicory (*Cichcorium intybus*), caraway (*Carum carvi*), birdsfoot trefoil (*Lotus corniculatus*), chervil (*Anthriscus cerefolium*), plantain (*Plantago lanceolata*), lucerne (*Medicago sativa*), and melilot (*Melilotus officinalis*). All herb species, except lucerne and caraway, were most competitive in the first harvest year. The proportion of all herbs, except lucerne, was higher at a 6-cut than at a 4-cut strategy, and application of cattle slurry also affected the competitiveness of the herbs. In general, lucerne, chicory, caraway and plantain were the strongest competitors; salad burnet and birdsfoot trefoil were intermediate; and melilot, fenugreek, and chervil were very weak competitors.

Keywords: herbs, competitiveness, temporary grassland, cutting, slurry

## Introduction

The utilisation of herbs in temporary grassland seed mixtures is related to their expected beneficial side-effects such as increased concentrations of essential macro- and microminerals in the herbage (Pirhofer-Walzl *et al.*, 2011). However, successful establishment and growth of forage herbs are important for farmers to implement their use; and central to this is the competitive ability of the herb species. As the traditional sown grassland species, perennial ryegrass and white clover, are highly competitive, herbs need to find their own niche in the struggle for survival (Søegaard, 2009). Thus, in multispecies grasslands the proportion of different herb species may vary considerably due to low competitiveness of some herbs (Søegaard *et al.*, 2008). Consequently, to examine the possibility for increasing the competitiveness of herbs in temporary grasslands, an experiment investigating the effects of seed mixture composition, cutting frequency and slurry application on the competitiveness was set up.

## Materials and methods

Different seed mixtures were established in spring 2008 on a sandy loam at the Research Farm Foulumgaard. These were undersown in spring barley (*Hordeum vulgare*). The 100% herb mixture (seed in percentage of total shown in brackets) comprised salad burnet (19.1), fenugreek (19.1), chicory (9.6), caraway (9.6), birdsfoot trefoil (9.6), chervil (9.6), plantain (4.8), lucerne (9.6), and melilot (9.6). The 5% and 50% herb mixtures included the same herb mixture, but it was sown in combination with a mixture of perennial ryegrass and white clover making up the remaining 95% and 50%, respectively. The total seeding rate was 25 kg ha<sup>-1</sup>. The experiment comprised plots of  $3 \times 8$  m, and it was set up with

three variables: 1) the proportion of herb seeds in the total seed lot was 5, 50 or 100%, 2) the cutting frequency was either 4 or 6 times per year, and 3) cattle slurry was applied at two levels equivalent to 0 or 200 kg total N ha<sup>-1</sup> (120 N in spring, and 80 kg N after cut 2 or cut 3 for the 4- and 6-cut strategies, respectively). Two replicates of each treatment were established. Slurry application was main plots, seed mixture was split plots, and cutting frequency was split-split plots. Plots were harvested by a Haldrup plot-harvester and the botanical composition was determined by hand separation of subsamples in spring and summer cuts (cut 3 in the 4-cut strategy and cut 4 or 5 in 6-cut strategy).

#### **Results and discussion**

The total dry matter yields increased in all three years by inclusion of herbs in the seed mixture when cut 4 times (Table 1). The opposite occured under the 6-cut strategy. Further, the yield stayed high for the 100% herb mixtures cut 4 times per year during all three years, whilst it was significantly reduced over years in the case of the 6-cut strategy.

Table 1. Dry matter yield (t DM ha<sup>-1</sup>) of the three grassland seed mixtures in the years 2009, 2010, and 2011. Values with different letters are significantly different (P < 0.05) within each column

Cuts per year		4						6				
Slurry applica-		0			200			0			200	
tion (kg total N ha-1)		0			200			0			200	
Year	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
5% herbs	12.5 <sup>b</sup>	9.6 <sup>b</sup>	8.9 <sup>b</sup>	13.5	10.8	9.5 <sup>b</sup>	11.8 <sup>a</sup>	$10.0^{a}$	8.9 <sup>a</sup>	12.6 <sup>a</sup>	10.7 <sup>a</sup>	8.8 <sup>a</sup>
50% herbs	14.1ª	10.9 <sup>ab</sup>	$10.8^{ab}$	14.7	11.5	11.0 <sup>ab</sup>	11.6ª	$10.0^{a}$	8.3ª	12.6ª	10.7ª	8.9ª
100% herbs	13.8ª	11.9 <sup>a</sup>	13.0 <sup>a</sup>	14.1	11.5	12.6ª	9.1 <sup>b</sup>	$6.8^{b}$	5.6 <sup>b</sup>	9.7 <sup>b</sup>	7.2 <sup>b</sup>	6.7 <sup>b</sup>

All herbs established well. However, chervil and fenugreek were quickly outcompeted and they are therefore not included in Table 2. In general, the herb species, except lucerne and caraway, were most competitive in the first harvest year. Throughout the year, i.e. spring vs. summer cuts, variations in the amount of herb species existed. Species such as salad burnet, chicory and melilot made up a significantly higher proportion of the total dry matter in spring compared to summer (P < 0.05). On the other hand, lucerne and birdsfoot trefoil were present in larger proportions in summer compared to spring. The proportion of most herbs, except for lucerne were higher in a 6- than in a 4-cut strategy. Further, the results

Table 2. Species composition in the herbage (percentage of total dry matter). Values with different letters are significantly different (P < 0.05) when comparing across mixture, slurry application, year, cutting strategy and time of year for the individual species

Species	% of see	herbs i ed mixt	in the ure	Slu applio (kg N	urry cation ( ha <sup>-1</sup> )		Year		Cuts p	er year	Time	of year
	5	50	100	0	200	2009	2010	2011	4	6	Spring	Summer
S. burnet	0.1 <sup>b</sup>	0.5 <sup>b</sup>	3ª	1.0	1.1	1.4	0.8	1.0	0.7	1.4	1.6 <sup>a</sup>	0.5 <sup>b</sup>
Chicory	2 <sup>b</sup>	10 <sup>b</sup>	19 <sup>a</sup>	11	10	21ª	4 <sup>b</sup>	1.2 <sup>b</sup>	10	11	12ª	9 <sup>b</sup>
Caraway	$0.7^{b}$	3 <sup>b</sup>	13 <sup>a</sup>	3	8	0.7	7	12	5	7	6	5
B. trefoil	$0.1^{b}$	0.9 <sup>b</sup>	$7^{a}$	3	2	3	2	2	1.6 <sup>b</sup>	4 <sup>a</sup>	2 <sup>b</sup>	3ª
Lucerne	$1.0^{b}$	20 <sup>b</sup>	42ª	25	17	17	25	22	33ª	8 <sup>b</sup>	19	23
Melilot	0.0	0.2	0.7	0.3	0.3	0.7	0.0	0.0	0.3	0.3	0.5ª	0.1 <sup>b</sup>
Plantain	$1.1^{b}$	5 <sup>b</sup>	$8^{\rm a}$	4	5	$8^{\rm a}$	3 <sup>b</sup>	$1.8^{b}$	3 <sup>b</sup>	ба	4 <sup>b</sup>	5ª
W. clover	53ª	31 <sup>b</sup>	$0^{c}$	31	25	30	29	24	24 <sup>b</sup>	32ª	23 <sup>b</sup>	33ª
P. ryegrass	42ª	29 <sup>b</sup>	$0^{c}$	18 <sup>b</sup>	28ª	17	27	29	21 <sup>b</sup>	26ª	29ª	18 <sup>b</sup>
Unsown	0.3 <sup>b</sup>	1.4 <sup>b</sup>	$8^{\rm a}$	3	3	1.6	4	6	1.4 <sup>b</sup>	5ª	3	4

indicated that application of cattle slurry affected the competitiveness of the herbs although differences were not significant.

Figure 1 highlights the interaction between the three dominating herb species (lucerne, caraway and chicory) in the 100% herb mixture without application of nitrogen. It is evident that the amount of chicory is reduced throughout the years in both the 4- and 6-cut strategy. The opposite is true for caraway, whilst the performance of lucerne peaks in the second year.



Figure 1. The percentage of lucerne, caraway and chicory in spring cuts over three harvest years in the treatment with 100% herbs in the seed mixture for the two cutting strategies. No slurry has been applied

#### Conclusions

The botanical composition was highly affected by management, i.e. amount of herbs in the seed mixture, cutting frequency and slurry application. This presents the farmer with an opportunity to manipulate the botanical composition in temporary multispecies grasslands in order to achieve desired beneficial effects.

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#### References

Pirhofer-Walzl K., Søegaard K., Høgh-Jensen H., Eriksen J., Sanderson M.A., Rasmussen J. and Rasmussen J. (2011) Forage herbs improve mineral composition of grassland herbage. *Grass and Forage Science* 66, 3, 415–423.

Søegaard K. (2009) Urter har mange funktioner i græsmarken [Herbs have many functions in the grass field]. Landbrugsavisen, 14 August 2009, p. 20.

Søegaard K., Eriksen J. and Askegaard M. (2008) Herbs in grasslands – effect of slurry and grazing/cutting on species composition and nutritive value. *Grassland Science in Europe* 13, 200–202.

## Establishment of switchgrass in permanent grassland

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## Abstract

The productivity of grassland in Central Europe follows a typical seasonal pattern, with maximum growth rates in spring and a decrease in net production in mid-summer. In contrast to  $C_3$  plants,  $C_4$  grasses show slow recovery after winter and highest productivity in summer. For bioenergy use, integrating warm-season grasses can be a method of enhancing dry matter production of permanent grassland. But due to the slow growth of C<sub>4</sub> grasses at the beginning of the vegetation period, competition from the grassland sward is strong. The aim of our studies performed at the experimental field of the University of Hohenheim (south-west Germany) was to investigate the establishment success of switchgrass (Panicum virgatum L.) in permanent grassland. Two varieties of switchgrass (lowland and upland types) were established by planting rhizomes and seedlings and by strip-seeding. As pre-treatments of the sward, spraying with total herbicide, soil tillage in bands with a rotary cultivator, and an untreated control were compared. Results showed poor growth of switchgrass from rhizomes in all plots. Growth of the planted seedlings was influenced by competition from the sward. Maximum plant height was reached after herbicide application and only small plants grew in the undisturbed grassland. Germination of seeded switchgrass was problematic and the growth rate was strongly regulated by sward competition.

Keywords: Panicum virgatum, bioenergy, permanent grassland, C<sub>4</sub> grasses

## Introduction

Due to changes in livestock farming there are more and more grassland areas no longer used for fodder production. Bioenergy production can be an alternative utilization for grassland biomass (Prochnow *et al.*, 2009). Conversion of free grassland into cropland has the effect of greatly reducing the soil carbon stock and in addition endangers biodiversity. For this reason, in many cases this conversion is unwanted or even forbidden. Furthermore, grassland areas are often on slopes and their soil conditions are not suitable for profitable field crops. When producing biomass for anaerobic fermentation or combustion, the principal aim is to obtain a maximum dry matter (DM) yield.

The characteristic growth rate of grassland exhibits a maximum in spring and lower productivity in summer when water availability is restricted.  $C_4$  plants, in contrast, develop slowly in spring and show higher growth rates with rising temperatures in summer due to a better water-use efficiency. Combination of the  $C_3$  grassland plants with  $C_4$  warmseason grasses can thus be a method of enhancing dry matter yields.

Switchgrass is a North American perennial  $C_4$  grass with genotypes adapted to different climate zones. The available genotypes have a wide range of growth characteristics and cold tolerance and can be subdivided into highland and lowland types. Switchgrass has a deep rooting system, the capacity for high DM yields and high water- and nitrogen-use efficiency. Traditionally switchgrass has been part of pasture vegetation in the plains and its potential as a herbaceous bioenergy plant is currently being investigated in North America and Europe (Lewandowski *et al.*, 2003).

Switchgrass is usually established in arable fields by seeding, but there are often difficulties with a high degree of dormant seeds and weed competition. Therefore, establishment of switchgrass in permanent grassland by sod-seeding presents a special challenge. The objective of this study was to investigate alternatives for the establishment of switchgrass in meadows without destroying the sward.

## Materials and methods

In a permanent grassland area with 65% grasses (relevant species: *Alopecurus pratensis*, *Lolium perenne*, *Poa trivialis*, *Holcus lanatus*), 20% legumes (*Trifolium pratense*, *Vicia sepium*, *Trifolium repens*) and 15% forbs (*Taraxacum officinale*, *Rumex acetosa*, *Crepis biennis* etc.) a field experiment was established in May 2011 after cutting the first growth. Three different pre-treatments of the sward were compared: spraying with a total herbicide (glyphosate), soil tillage in bands (20 cm wide, 3–5 cm depth) and an untreated control (rows marked by small rill). Seeds, seedlings and rhizomes of an upland type of switchgrass (cv. Shawnee) and a lowland type (cv. Kanlow) were planted in rows 75 cm apart. Between the rows, a strip of 50 cm width was cut frequently with a lawn mower to limit the effects of competition from the sward on the young plants and to mark the rows. The remaining 25-cm wide strips with the switchgrass were left uncut during the growing period.

The experiment was arranged in a block design with 4 replications and a plot size of 5 m<sup>2</sup>. The site was located at Stuttgart-Hohenheim (48.7137°N, 9.2122°E, altitude 400 m a.s.l., mean annual rainfall 698 mm, mean daily temperature  $8.8^{\circ}$ C) on a silty loam soil. The development of the switchgrass plants was investigated at the end of July.

Additionally, the germination rate of the seeds was tested at 20°C without stratification and after a one-day and a 14-day long stratification at 3°C.

Plant growth data were evaluated statistically using SAS MIXED procedure (SAS 9.3).

#### **Results and discussion**

Germination of the switchgrass seeds was very poor (Table 1). Only cv. Shawnee showed better germination after a 2-week stratification, whilst this treatment had a negative effect on cv. Kanlow. Problems with seed dormancy are well-known and various stratification methods can be applied, but success is not guaranteed and there appear to be differences between cultivars (Treseler, 2007).

Table 1. Germination rate of two switchgrass cultivars after different stratification period
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		cv. Shawnee	e		cv. Kanlow	7
Stratification period (days)	0	1	14	0	1	14
Germination rate (%)	7	7	25	10	5	7

Seeding of switchgrass in an undisturbed sward was not very successful (Table 2). Less than 0.5% of the viable seeds germinated and grew, and also the achieved plant height was only 10 cm due to competition from the grassland sward. Neither was soil tillage in bands effective, because the grassland plants recaptured this area soon after the procedure. Germination and growth of young switchgrass plants was therefore too slow to benefit greatly from strip tillage. When the sward was destroyed by herbicide, survival and plant size increased significantly. In these plots there were no living grassland plants at seeding time. In the course of time other plants established from the natural seed reservoir and a grassland-like vegetation re-established. The dominant species here was dandelion (*Taraxacum officinale*). A successful method for establishing switchgrass is the planting of seedlings. Even though

competition from the sward was noticeable, the differences between pre-treatments were not significant because the period with slow germination and growth here was in a greenhouse – therefore without competition – and not in the field. Only a few plants established from rhizomes. The rhizomes of switchgrass are small and not as robust as rhizomes of other  $C_4$  grasses such as miscanthus. The loss rate was high, with no plants left in some plots. In this experiment, establishment of switchgrass from rhizomes was not an effective technique. In contrast, Treseler (2007) attained good results with rhizomes planted in late autumn. Differences between the two tested cultivars were not very pronounced, but the highland type (Shawnee) always showed higher survival rates. Highland types are also known to be less sensitive to higher cutting frequencies and therefore more appropriate when the biomass is used for biogas production.

In agricultural practice sod-seeding can be an easy and cost-effective system, but slow germination and a lack of competitive ability of young plants are the main obstacles to successful establishment of perennial  $C_4$  grasses in  $C_3$  grassland (Doll *et al.*, 2011).

Establishment by	Pre-treatment	Surviv	val (%)	Plant size (cm)
seeding/planting	of sward	cv. Shawnee	cv. Kanlow	(mean of cultivars)**
Seeds	without	$0.45\pm0.27$	$0.33\pm0.18$	10.08 <sup>ab</sup>
	tillage in bands	$0.38\pm0.24$	$0.30\pm0.18$	8.04 <sup>b</sup>
	total herbicide	$2.38\pm0.28$	$1.60\pm0.08$	39.03°
Rhizomes	without	$2.38 \pm 1.37$	0	13.16 <sup>abd</sup>
	tillage in bands	$7.14 \pm 2.38$	$5.95 \pm 4.51$	21.40 <sup>ae</sup>
	total herbicide	$9.52 \pm 3.37$	$2.38 \pm 1.37$	37.16 <sup>c</sup>
Seedlings	without	$54.76 \pm 9.82$	$28.33 \pm 3.19$	26.93 <sup>cde</sup>
	tillage in bands	$46.43 \pm 4.90$	$35.00 \pm 9.95$	29.28 <sup>ce</sup>
	total herbicide	$79.76 \pm 7.11$	$48.33 \pm 8.77$	34.35°

Table 2. Survival rate (percentage of planted seedlings, rhizomes or viable seeds, mean values and s.e.\*) and plant size of switchgrass 10 weeks after seeding/planting

\* for this dataset ANOVA was not applicable,

\*\* means of cultivars are shown since threefold interaction of treatments is not significant.

#### Conclusions

The experiment showed that establishment of switchgrass in grassland can be achieved by planting seedlings and, to a lesser extent, by seeding, but only when competition from the sward is suppressed. In a next step, management of these mixed plant communities has to be optimised and the variation of botanical composition with time needs to be analysed. Thus, further investigations are required before the anticipated increase in dry matter yield through the introduction of switchgrass in grassland can be evaluated.

#### References

Doll J.E., Haubensak K.A., Bouressa E.L. and Jackson R.D. (2011) Testing disturbance, seeding time, and soil amendments for establishing native warm-season grasses in non-native cool-season pasture. *Restoration Ecology* 19, 1–8.

Lewandowski I., Scurlock J.M.O., Lindvall E. and Christou M. (2003) The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy* 25, 335–361. Prochnow A., Heiermann M., Plöchl M., Linke B., Idler C., Amon T. and Hobbs P.J. (2009) Bioenergy from permanent grassland – A review: 1. Biogas. *Bioresource Technology* 100, 4931–4944.

Treseler C.H. (2007) Optimierung ausgewählter Biomasseparameter von Switchgrass (Panicum virgatum) für die industrielle Verwertung. Dissertation Universität Bonn, IBL Bonn, 147 pp.

# Influence of pedoclimatic and management factors on botanical and functional composition of grasslands

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## Abstract

Permanent grasslands deliver several agronomic and environmental services that are strongly, but not only, linked to vegetation composition. Vegetation composition can be described by several criteria that can be classified in terms of botanical (presence, dominance) and functional (reproduction, survival, dissemination strategies) features of species. We aimed to estimate the role of pedoclimatic and management factors on these features by setting predictive models. 190 permanent grasslands were studied on a large gradient of conditions in France. Functional composition (FC) was assessed through leaf dry matter content, date of flowering, oligotrophic species richness, and proportions of entomophilous species, and of grasses, legumes and forbs. Botanical composition (BC) was mainly influenced by pedoclimatic factors, whereas FC was influenced by pedoclimatic and management factors. Interactions between factors have to be taken into account to predict the characteristics of vegetation. The number of factors for a correct prediction of BC is more important (11 to 12) than for FC (2 to 7), and the prediction quality of models shows differences between BC and FC. Finally, it appears that BC and FC cannot be predicted from the same factors or combinations of factors.

Keywords: biodiversity, soil, climate, France

## Introduction

Various characteristics of grassland vegetation are used to predict the ecosystem services delivered by permanent grasslands. These features are highly influenced by pedoclimatic and management factors, but contrasting results have been obtained. We aimed to test the relation between vegetation characteristics and their predicting factors, for a broad range of pedoclimatic and management conditions, considering simultaneously the botanical composition (BC) and functional composition (FC) of swards. Despite the wide range of pedoclimatic and management situations found in France (representative of most farm situations), the very high biodiverse grasslands (more than 60 species) were poorly represented in our network. Results are therefore interpreted against this background.

## Materials and methods

A set of 190 permanent grasslands on 78 farms was selected to cover the main French pedoclimatic areas from Atlantic to Alpine conditions (from sea level to 2000 m altitude) and the most frequent management situations. The management gradient was defined by a diversity of farm systems (dairy or beef cattle, sheep) and management practices (type and number of uses (grazing, cutting), type of animals and livestock stocking rate, type and amount of fertilizers). Further details about these grasslands are described by Michaud *et al.* (2011). For each grassland, the BC was determined once in spring 2009. A complete list and dominance of species (visual estimation in 8 quadrats of  $50 \times 50$  cm), and functional composition (FC) was determined by i) the mean plot values from BC, and ii) species features in eFLORAsys database (Plantureux *et al.*, 2010). FC includes the proportion of grasses, legumes and forbs; entomophilous species; number of oligotrophic species, date of flowering, and three mean plant traits: leaf dry matter content (LDMC), specific leaf area (SLA) and leaf life span (LLS). Because of high correlations between these traits, we only used LDMC in the data analyses. We asked the farmers how they had managed their plots during the previous five years: mineral and organic fertilization (N, P, K), annual number, dates and types of use (cuts, grazing periods), grazing intensity (livestock mean and instantaneous stocking rates). From the national network METEO-France® (information for each  $10 \times 10$  km), we calculated the ten-day periods, monthly and annual temperature, radiation, rainfall and evapotranspiration. Soil parameters were obtained either from farmers and advisers of extension services (type of soil, soil bearing capacity, slope and humidity of soil) or calculated from BC by Ellenberg indices (soil fertility, acidity, humidity). Statistical relations between vegetation features and pedoclimatic or management factors were established with a stepwise regression (SAS package®) for quantitative variables (FC) and with a canonical correspondence analysis (CANOCO software®) for qualitative variables (BC).

#### **Results and discussion**

BC was clearly linked to pedoclimatic factors, especially for species present (Table 1). The only management factor influencing dominance or presence of species was PK fertilization, which improves soil fertility (considered as a pedoclimatic factor). Compared to BC, FC appears to be more related to management factors. The annual number of uses is the most frequent factor, and it can be either the number of cuts or of animal grazing periods. This variable is also closely linked to the first date of use in spring, resulting in a global variable assessing the earliness and the frequency of use. BC is explained by more factors than FC. This can be explained by i) by the very broad range of pedoclimatic conditions of the network, reducing in proportion the influence of management, and ii) the fact that presence of particular species also depends on the regional list of present species (habitat specificity). Different species sharing the same functional features can be found under different pedoclimatic conditions. Most of the factors explaining BC are in fact interactions between factors, especially indicating that the impact of management on BC highly depend on pedoclimatic conditions, in accordance with Klimek *et al.* (2007).

The overall quality of the models is quite good, indicating that all the variables taken into account are relevant predictors of BC and FC. Nevertheless, main factors (statistically selected according to their *P* values in the models) explain from 9% (for legumes proportion) to 73% (number of oligrotrophic spp.) of the variability of FC. This means that FC is difficult to predict with a limited number of parameters.

## Conclusions

When tested across a wide range of situations, grassland botanical and functional vegetation characteristics were not strictly driven by the same pedoclimatic and management factors. Numerous factors have to be taken into account, and interactions between factors also considered. These results lead to interesting perspectives with respect to optimization of ecosystem services. Adapting management features to pedoclimatic conditions provides a means of improving selected BC or FC features that are highly related to these services.

Table 1. Statistical relations between botanical and functional characteristics and pedoclimatic and management factors of a set of 190 French permanent grasslands. Stars (\*) indicate interactions. Factors are sorted according to their appearance rank in statistical models (W): during winter (15 Oct to 15 Apr) – (S): during summer (15 Apr to 15 Oct)

Characteristics of vegetation: Botanical (BC) or Func- tional (FC) Com- position	R <sup>2</sup> of the model	R <sup>2</sup> of main fac- tors	Main pedoclimatic factors	Main manage- ment factors
Presence or ab- sence of species (BC)	_	_	Average temperature (W) and (S), Annual radiation, + numerous interactions involving annual radiation and precipitations, aver- age soil evapotranspiration, soil fertility and <u>acidity</u> Date of first use*Soil humidit	Total K fertili- zation * Total P fertili- zation
Dominance of species (BC)	_	_	Average temperature (S) + numerous interactions involving Annual radiation, average soil evapotranspi- ration, type of soil, soil fertility and acidity, soil bearing capacity and total K fertilization	<u>,                                     </u>
Grasses propor- tion (FC)	0.75	0.23	Soil humidity	Number of uses, Mineral N fertilization
Legumes propor- tion (FC)	0.76	0.09		Total K ferti- lization, Num- ber of uses
Forbs propor- tion (FC)	0.79	0.19	Annual rainfall	
Date of flower- ing (FC)	0.85	0.47	Average temperature (W), Soil humidity, Soil fertility, Average temperature (S), An- nual precipitations	Number of uses, Organic N fertilization
Number of oli- gotrophic species (FC)	0.92	0.73	Soil fertility, Soil acidity *Average tem- perature (W), Average soil evapotranspira- tion (W)	
Percentage of entomophilies species (FC)	0.77	0.30	Soil humidity	Number of uses, Organic N fertilization, Mineral N fertilization
Leaf dry mat- ter Content (FC)	0.85	0.31	Soil fertility, Annual radiation	Mean livestock density

#### References

Klimek S., Kemmermann A.R.G., Hofmann M. and Isselstein J. (2007) Plant species richness and composition in managed grasslands: the relative importance of field management and environmental factors. *Biological Conservation* 134, 559–570.

Michaud A., Plantureux S., Amiaud B., Carrere P., Cruz P., Duru M., Dury B., Farrugia A., Fiorelli J-L., Kerneis E. and Baumont R. (2011) Identification of the environmental factors which drive the botanical and functional composition of permanent grasslands. *Journal of Agricultural Science*, Available on CJO 2011 doi:10.1017/S0021859611000530.

Plantureux S. and Amiaud B. (2010) e-FLORA-sys, a website tool to evaluate the agronomical and environmental value of grasslands. *Grassland Science in Europe* 16, 732–734.

## Establishment of Italian ryegrass by self-seeding in crop-livestock systems

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## Abstract

The aim of this study was to evaluate the influence of pasture/crop rotations in the establishment of Italian ryegrass by self-seeding. Lambs grazed Italian ryegrass under rotational or continuous stocking and under low and moderate grazing intensities. In summer, the paddocks were divided and sown with soybean or maize. The experimental design was a completely randomized blocks, arranged in a factorial  $(2\times2\times2)$ , with four replicates. Italian ryegrass establishment by self-seeding was evaluated by tillering and by forage mass assessments, before and after summer crop harvest. Stocking methods and grazing intensities had no influence on pasture establishment. Tiller density and forage mass differed according to crop rotation (higher in soybean areas) and evaluation periods. Pasture establishment was faster in soybean rotations.

Keywords: mixed systems, maize, soybean, Lolium multiflorum

## Introduction

The use of soybean (*Glycine max* L.) and maize (*Zea mays* L.) crops in rotation with Italian ryegrass (*Lolium multiflorum* Lam.), established by self-seeding, is widespread in southern Brazil. Farmers use this system because the emergence of seedlings begins before crop harvest, the pasture growing beneath the crop canopy. This method incurs no pasture establishment costs, and anticipates forage production for the winter period.

Tillering is a way of ensuring grass survival and persistence, and is influenced by management and several environmental factors. A satisfactory and fast pasture establishment is extremely dependent on the soil seed bank and the summer crop influence on tillering. The summer crops could compete with grass tillers at the beginning of their establishment for light, water and nutrients, which could delay or prevent the initial tillering and the subsequent pasture establishment. This study has aimed to evaluate the establishment of Italian ryegrass by self-seeding in integrated crop-livestock systems.

## Materials and methods

The experiment was conducted at the Federal University of Rio Grande do Sul, Brazil  $(30^{\circ}05'22'' \text{ S}, 51^{\circ}039'08'' \text{ W})$ . The experimental design was a completely randomized block, arranged in a factorial design (2 stocking methods  $\times$  2 grazing intensities  $\times$  2 summer crops), with four replicates (plots measuring 0.2 to 0.4 ha). Since 2003 the Italian ryegrass rotates with soybean or maize, and has been managed with rotational or continuous stocking with herbage allowances of 2.5 or 5 times (moderate and low grazing intensities, respectively) the intake potential of lambs, considered as 4% of LW (NRC, 1985). The rotation cycle was defined by Italian ryegrass leaf lifespan. In summer, each paddock was divided and sown with soybean or maize. Data refer to 2009 and 2010 assessments. The stocking season was from 15 September to 15 November, 2009. The soybean and maize were sown on 29 January 2010 and harvested on 1 June and 16 June, respectively. Pasture establishment by self-seeding was evaluated by tiller density and forage mass. Tillers were assessed by tagging coloured
rings (Carvalho *et al.*, 2001), which were evaluated twice a month between 1 May and 28 July 2010. The forage mass was evaluated by cuts at ground level with a square metallic frame (0.25 m<sup>2</sup>) on 31/5, 11/7 and 28/7/2010. The pasture boundary line was made according to Matthew *et al.* (1995). Since tiller densities and forage mass were not evaluated on the same date, for the correlation between forage mass tiller density (Figure 1), tiller density data from previous dates of the forage mass evaluations were used. Analyses of variance were performed at P < 0.5 significance using Mixed Procedures (SAS, 2002). The model used included fixed effects of blocks, summer crops, stocking methods and grazing intensities and random effects of date and the interactions of summer crops × stocking methods × grazing intensities × date. The measurement periods were considered to be repeated measures in time. When differences between treatments were detected, the treatments were compared using the Tukey test (P < 0.05).

Table 1. Tiller density and forage mass from establishment of Italian ryegrass (*Lolium multiflorum* Lam.) by self-seeding before and after soybean and maize harvest in different months of evaluation in integrated crop-livestock system

Crop		May	June	July
Maize	Tiller density	6143.1Aa	5318.6ABb	6803.2Ab
Soybean	(Tiller m <sup>-2</sup> )*	5616.1Ba	8799.4Aa	9548.3Aa
Maize	Forrage mass	460.0E	674.4D	853.75C
Soybean	(kg DM ha <sup>-1</sup> )**	347.50E	1210.0B	1511.0A

\*Means followed by same letter of lower case in column and upper case in row do not differ (P > 0.05) according to Tukey test.

\*\*Means followed by same letter in column and in row do not differ (P > 0.05) according to Tukey test.



\* .....after maize; - - - after soybean; -- boundary line;

Dates of evaluation:  $\triangle 29/5/2010$ ;  $\bullet 11/7/2010$ ;  $\blacksquare 28/7/2010$ .

Figure 1. Forage mass/tiller density relationships during establishment of Italian ryegrass (*Lolium multiflorum* Lam.) by self-seeding before and after soybean and maize harvest in integrated crop-livestock system. (a) Continuous stocking with low grazing intensity; (b) Continuous stocking with moderate grazing intensity; (c) Rotational stocking with low grazing intensity; (d) Rotational stocking with moderate grazing intensity

#### **Results and discussion**

The stocking methods and grazing intensities did not interfere in tiller density and in forage mass (P > 0.05). Both the tiller density and forage mass of Italian ryegrass differed between the crops and among the evaluation periods (Table 1). Initially, in May, the tiller density and the forage mass were similar in the areas with soybean and maize. In the following months (June and July), the tiller density and the forage mass were higher in areas sown with sovbean. Figure 1 illustrates these results, where the points of the areas with sovbean approach more quickly from the boundary line than the areas sown with maize. Thus, the environmental conditions in the soybean areas favoured an increase in tiller numbers and in mass per tiller. Tillering is influenced by several factors; however, in the present study, the effect of light quality in the pasture canopy was probably more evident, although light interception was not evaluated. The light red: far-red restriction decrease tillering (Casal et al., 1985), interfering directly in the forage mass, which is a product of the tiller density and mass per tiller. During the process of soybean maturity in May, leaf fall occurred and then the tillers could gain access to light. After the soybean harvest, tillers had full access to light, which provided an increase in the tiller density and in forage mass. However, in the maize maturity process the leaves do not fall, they just senesce. Therefore, the tillers had restricted light access during corn maturity process. Photosynthesis is limited in shaded sites; thus tiller reduction can be associated, in part, to the allocation of current photosynthate to existing tillers rather than to new tiller buds (Devkota *et al.*, 1998). Because the light restriction in tillers occurred in areas with maize, the pasture was established later in maize fields than in fields with soybean.

#### Conclusions

The stocking methods and grazing intensities, managed in the previous year, do not interfere in the establishment of Italian ryegrass by self-seeding. Regardless of the choice of summer crop soybean or maize – Italian ryegrass is established satisfactorily by self-seeding; however, in areas sown with soybean the Italian ryegrass established faster.

#### References

Casal J.J., Deregibus V.A. and Sánchez R.A. (1985) Variations in tiller dynamics and morphology in *Lolium multiflorum* Lam. vegetative and reproductive plants as affected by differences in red/red far-red irradiation. *Annals of Botany* 56, 553–559.

Carvalho C.A.B., Da Silva S.C., Sbrissia A.F., Pinto L.F.M., Carnevalli R.A., Fagundes J.L. and Pedreira C.G.S. (2000) Demografia do perfilhamento e taxas de acúmulo de matéria seca em capim 'Tifton 85'sob pastejo. *Scientia Agricola* 57, 591–600.

Devkota N.R., Kemp P.D., Valentine I. and Hodgson J. (1998) Performance of perennial ryegrass and cocksfoot cultivars under tree shade. *Proceedings of the Agronomy Society of New Zealand* 28, 129–135. Matthew C., Lemaire G., Sackville-Hamilton N.R. and Hernandez-Garay A. (1995) A modified self-thinning equation to describe size/density relationships for defoliated swards. *Annals of Botany* 76, 579–587.

National Research Council – NRC (1985) Nutrient requirement of sheep. 6th ed. Washington, D.C.: National Academy of Science, 99 pp.

# Yielding of grass cultivars in pure stands and phenologically different meadow mixtures in the first year of utilization

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## Abstract

The objective of this study was to estimate yielding of fourteen Polish cultivars of three tallgrowing grass species – *Dactylis glomerata, Festuca pratensis, Phleum pratense* and three shortgrowing grass species – *Lolium perenne, Festuca rubra* and *Poa pratensis*, in pure stands and two mixtures with different heading dates. The studies were carried out in central Poland in 2011 on a mineral brown soil. The experiment was designed as a randomized complete block with four replications. Three cuts were made with the first one when the individual cultivars in pure stands and the dominating cultivars in the mixtures headed. *Dactylis glomerata* cv. Amera and the cultivars of *P. pratensis* headed first, whereas the cultivars of *Ph. pratense* were the latest (13 days of difference). There were significant differences in DM yield – larger between species and smaller between cultivars. The yields among cultivars of tall grasses were similar, while greater differences were observed between cvs of short grasses. The productivity of the early mixture was slightly better than the mid-early on all harvests. The yield of mixtures was determined by *L. perenne, F. pratensis* and *D. glomerata* dominated in sward.

Keywords: earliness, grass cultivars, meadow mixtures, pure stands, yields

## Introduction

Earliness is one of the important characters in grass breeding (Laidlaw and Reed, 1993). In Poland not all grass species are adequately differentiated in earliness of cultivars. It is crucial to ensure both persistence and productivity of grass mixtures. Therefore, studies of the biological development and production of grass cultivars are still necessary (Rutkowska *et al.*, 1997). The aim of this study was to estimate the dry matter (DM) production of 14 Polish cultivars of six grass species in pure stands and in two meadow mixtures with different earliness of heading.

## Materials and methods

The experiment was undertaken in central Poland at the Experimental Station SGGW in 2011 on a mineral brown soil. The trial was arranged in a randomized complete block. The content of available phosphorus, potassium and magnesium were medium and soil pH<sub>KCl</sub> was 5.2. Mineral fertilizer was applied at 180, 26 and 100 kg ha<sup>-1</sup> of NPK per year, respectively. Polish cultivars of six grass species – *D. glomerata* (Amera, Berta), *F. pratensis* (Pasja, Wanda), *Ph. pratense* (Pasja, Wanda), *L. perenne* (Bajka, Diament, Gagat, Naki), *F. rubra* (Reda, Kos) and *P. pratensis* (Eska-46, Skiz) were sown in pure stands and in two meadow mixtures with different earliness. The early mixture consisted of cvs – Amera (10%), Wanda (30%), Skala (20%), Naki (20%), Kos (10%) and Eska-46 (10%) while the mid-early (in the same proportions) – Berta, Pasja, Karta, Bajka, Reda and Skiz. Three cuts were made: the first at the heading phase of the individual cultivars in pure stands and of cultivars dominating in the mixtures swards; the second on 28 July, and the third on 28 September. Cover, height of sward in the terms of cuts, yield (DM t ha<sup>-1</sup>) and botanical composition (proportions of sown cultivars in pure stands and mixtures) were determined.

Data were evaluated by one – factor analysis of variance and verified using LSD test (Statgraphics plus 4.1). The weather conditions during the vegetation were favourable for growth of grasses; the mean temperature (April-September) was 16.0°C and the sum of precipitation 495 mm (rainfall was moderate in spring but heavy in July – 295 mm).

#### **Results and discussion**

Differences in earliness of cultivars in pure stands were clearer than in mixtures (Rutkowska *et al.*, 1997). The span in the term of the first cut of examined cultivars was 13 days. The *D. glomerata* cv. Amera and cultivars of *P. pratensis* headed first and were cut on 25 May. Six days later (2 June) the mid-early *D. glomerata* cv. Berta and *F. rubra* cv. Reda were harvested, and then cultivars of *L. perenne* Bajka, Naki and *F. pratensis*, as well as the mixtures. The latest were cvs of *L. perenne* Diament and Gagat (5 days later than Bajka and Naki used in mixtures) and *Ph. pratense*. Results showed significant differences in DM yield; these were greater between the species and smaller between cultivars (Table 1). In pure stands the highest yields of tall-growing grass cultivars were obtained at the first cut and these determined the annual yields. The yields at individual cuts and annually among these grasses were similar.

Caracian	Cultinum		DM yiel	d (t ha <sup>-1</sup> )		Swar	Cover (%)		
Species	Cultivar	I cut	II cut	III cut	Annual	I cut	II cut	III cut	Mean
D alomanata	Amera	5.60	4.70a	4.29	14.60	69.8b	63.6a	56.3a	90.1a
D. giomeruia	Berta	6.44	4.82a	3.65	14.91	84.6a	63.0a	50.8ab	86.9abc
F pratonsis	Pasja	5.32	4.16ab	3.74	13.21	71.2b	45.8b	45.8b	84.3bc
r. praiensis	Wanda	6.33	3.79ab	3.77	13.87	68.3b	44.2b	45.4b	82.5c
Dh pratansa	Karta	6.62	3.19b	3.69	13.50	76.2ab	48.6ab	41.3b	87.4ab
Th. pratense	Skala	6.53	3.65ab	4.41	14.59	85.2a	46.2b	44.8b	86.4abc
LSD 0.05		ns	1.43	ns	ns	13.28	16.78	9.84	4.86
F. rubra	Reda	3.91b	4.42a	5.10ab	13.43ab	50.0c	45.6a	43.1a	87.5ab
	Bajka	6.31a	2.89b	5.31a	14.50a	54.4c	28.0d	35.6c	89.0ab
I noromno	Diament	7.51a	3.75ab	3.98bc	15.24a	64.9ab	37.6abc	38.5bc	82.9b
L. perenne	Gagat	7.82a	3.42ab	4.79ab	16.03a	72.1a	36.3bcd	39.2b	82.9b
	Naki	6.44a	3.10ab	4.37abc	13.91ab	56.5bc	28.8cd	36.2bc	86.1ab
P protonois	Eska46	2.73b	4.11ab	4.11abc	10.95b	51.3c	45.8a	45.4a	92.9a
r. praiensis	Skiz	3.47b	4.42a	3.05c	10.93b	52.4c	43.8ab	44.3a	91.5a
LSD 0.05		2.05	1.50	1.32	3.29	8.62	8.87	3.49	7.69
Mixturo	Early	6.88	4.36	2.81	14.05	74.7	54.1a	63.4	88.0a
witxtule	Medium early	5.95	3.78	2.75	12.49	66.7	49.8b	63.4	87.5ab
LSD 0.05		ns	ns	ns	ns	ns	4.10	ns	2.91

Table 1. DM yield (t ha-1), sward height (cm) and cover (%) of cultivars and mixtures

Values in columns with the same letters (a, b, c) create homogenous groups at 0.05 probability level.

Larger differences were observed between cultivars of short-growing grasses. The productivity of cultivars of *P. pratensis* and *F. rubra* was greater in the second and third cuts. The greatest annual yield was from cultivars of *L. perenne*, while *P. pratensis* had the lowest yields. The productivity of the early mixture was slightly better than the mid-early mixture, irrespective of cut. The contribution of weeds varied in cuts depending on the species and cultivar; the greatest amount was in *P. pratensis*, especially in the first cut (16–18%, Table 2). The smallest proportions of weeds were observed in the third cut, irrespective of cultivar. Changes in botanical composition of mixtures were noticeable at all harvests (Figure 1) due to competition between each component of the mixtures (Aldrich and Camlin, 1979; Borawska, 1997). Yields of mixtures were determined by the cultivars of *L. perenne*, *F. pratensis* and *D. glomerata* that dominated in the sward.

Species	Cultiver	I cut		II c	cut	III cut		
species	Cultival	Grasses (%)	Weeds (%)	Grasses (%)	Weeds (%)	Grasses (%)	Weeds (%)	
D. glomerata	Amera	98.2	1.8	99.8	0.2	99.7	0.3	
	Berta	97.5	2.5	99.9	0.1	100.0	0.0	
F. pratensis	Pasja	92.8	7.2	94.6	5.4	98.1	1.9	
	Wanda	99.2	0.8	99.3	0.7	99.9	0.1	
Ph. pratense	Karta	95.8	4.2	98.6	1.4	99.5	0.5	
	Skala	98.1	1.9	99.0	1.0	99.7	0.3	
F. rubra	Reda	93.2	6.8	97.6	2.4	98.2	1.8	
P. pratensis	Eska46	83.7	16.3	80.2	19.8	96.1	3.9	
	Skiz	82.0	18.0	95.8	4.2	95.2	4.8	
L. perenne	Bajka	97.5	2.5	98.3	1.7	99.4	0.6	
	Diament	98.3	1.7	99.4	0.6	99.7	0.3	
	Gagat	98.7	1.3	98.9	1.1	99.8	0.2	
	Naki	94.0	6.0	98.9	1.1	98.7	1.3	
Mixture	Early	97.7	2.3	99.7	0.3	100.0	0.0	
	Medium	98.9	1.1	99.6	0.4	99.8	0.2	

Table 2. Proportions of weeds in sown cultivars and mixtures (%)



Figure 1. Botanical composition of meadow mixtures (%)

## Conclusions

The range in heading dates among cultivars was 13 days. Yields per cut and total yield among cultivars of tall-growing grass species were similar. Larger differences were observed between cultivars of short-growing grasses. Productivity of the early mixture was slightly better than mid-early one, irrespective of cut. Cultivars of *L. perenne* (Naki and Bajka) were very aggressive components of mixtures in the first year of utilization.

## References

Aldrich D.T.A. and Camlin M.S. (1979) Experience of changes in sward composition and productivity from evaluation of species and varieties. *Proceeding of British Grassland Occasional Symposium* 10, 85–91.

Borawska B. (1997) Changes in species composition of meadow mixtures with differential earliness of grass cultivars in 3-year utilization. *Zeszyty Problemowe Postępów Nauk Rolniczych* 439, 341–349 (in Polish with English summary).

Laidlaw A.S. and Reed K.F.M. (1993) Plant improvement: the evaluation and extension process. *Proceedings of the XVII International Grassland Congress* 385–392.

Rutkowska B., Lewicka E. and Janicka M. (1997) Phenological differentiation of grass cultivars used in mixtures and pure stands. *Biuletyn Oceny Odmian* 28, 119–126 (in Polish with English summary).

# Ecophysiological model of growth of annual ryegrass as function of nitrogen fertilization

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## Abstract

Crop growth is modelled using a set of mathematical equations, based on physiological processes and environmental variables. The growth models may allow analysis of production components, providing an integrated overview of production. In order to adjust models of biomass accumulation as a function of photosynthetically active radiation absorbed (PAR<sub>a</sub>) in an annual ryegrass pasture (*Lolium multiflorum* Lam.), four N fertilization rates were applied (zero, 50, 100 and 200 kg ha<sup>-1</sup>) and arranged in a complete block design with three replicates. Grazing was done each time canopy intercepted 95% of incident light and stopped at 30% of interception. The biomass accumulation, as a function of PAR<sub>a</sub> during the establishment, was adjusted to linear plateau model for N<sub>zero</sub> and linear model for the other doses. In regrowth, there were also different models for N<sub>50</sub> (linear plateau), N<sub>100</sub> (linear) and N<sub>200</sub> (linear plateau). Biomass accumulation as a function of PAR<sub>a</sub> presented different models depending on sward condition (establishment and regrowth) and N fertilization. Therefore, the accumulation of biomass can be estimated using parameters describing the time-evolution of LAI, the absorption efficiency of PAR and the local ratio between the global solar radiation and PAR.

Keywords: grazing, light interception, *Lolium multiflorum*, photosynthetically active radiation, radiation balance, radiation use efficiency

## Introduction

Growth models may allow detailed analysis of production components and they can also provide an integrated overview of the production. The ability of plants to intercept and absorb photosynthetically active radiation (PAR), and turn it into energy for physiological processes and growth, are the basis for estimating the biomass accumulation (Thornley and Johnson, 2000). The aim of this study was to adjust a model of biomass accumulation as a function of PAR absorbed (PAR<sub>a</sub>) in annual ryegrass fertilized with different rates of N fertilizer.

## Materials and methods

The experiment was carried out at Estação Experimental Agronômica of UFRGS, Brazil (30°05'52" S, 51°39'08" W) on a Hapludult soil. Four annual rates of N (zero, 50, 100 and 200 kg ha<sup>-1</sup> of N, as ammonium sulphate as a single dose) were applied in a complete block design with three replications. Herbage mass (HM) and its N content were measured weekly (4/6/2008 to 12/11/2008). Photosynthetically active radiation (PAR) fluxes were continuously monitored: incident (PAR<sub>10</sub>), transmitted (PAR<sub>1</sub>), reflected by soil (PAR<sub>10</sub>) and reflected by

soil+crop (PAR<sub>rsc</sub>). The PAR<sub>a</sub> was calculated by the following equation (Varlet-Grancher *et al.*, 1989): PAR<sub>a</sub> = PAR<sub>inc</sub>+PAR<sub>rs</sub>–PAR<sub>rsc</sub>–PAR<sub>t</sub>. Grazing was performed during 2–4 hours by heifers and initiated when the light interception (LI) of swards were 95% and stopped at 30%. The results of total N in biomass were related to the respective values obtained for herbage mass and compared to the model proposed by Lemaire & Sallete (1984) for grasses with a metabolic pathway C3:% N = 4.8(HM)<sup>-0.32</sup>, where %N is the N content in aerial biomass; 4.8 N content (%) in the formation of the first tonne of HM; –0.32 coefficient of dilution of N in the growth period. According to the method of analysis of crop growth developed by Monteith (1972), it is possible to establish a linear relationship between the production of HM and the amount of PAR<sub>a</sub>, HM = RUE×PAR<sub>a</sub>, where RUE is radiation-use efficiency (regression coefficient). Data were submitted to regression analysis (*P* < 0.05). The parameters for biological interpretation of the models were compared using confidence intervals.

#### **Results and discussion**

Nitrogen concentration in the treatment without N fertilization (N<sub>rem</sub>) was always below that predicted by the model proposed by Lemaire & Salette (1984), limiting the rate of herbage mass accumulation and preventing the attainment of the critical LAI (95% LI). In contrast, values from N<sub>100</sub> and N<sub>200</sub> treatments during the establishment period were higher than predicted by the model, indicating that there was no growth limitation and that luxury consumption possibly occurred (Figure 1). In the  $N_{s_0}$  treatment there was some limitation at the beginning of establishment due to the lateness in the application of fertilizer. But there was a recovery later, and the values were also above those of the model (Figure 1b). In the regrowth phase, N content in herbage mass of  $N_{50}$  and  $N_{100}$  were lower than that predicted by the model, indicating that N probably limited growth during these periods (Figure 1). The restriction of N limits the development of leaf area, because it affects the emission rate of leaves, their life span and size (Lemaire and Chapman, 1996) which results in reduced LAI and LI. The treatment N<sub>zero</sub> was not grazed because it did not reach the proposed level of LI, whilst the N<sub>50</sub> could be grazed on one occasion (one regrowth). The treatments N<sub>100</sub> and N<sub>200</sub> provided two regrowths. Models of HM accumulation as a function of PAR<sub>a</sub> for the establishment phase were adjusted to a linear plateau model in N<sub>zero</sub>, while the response for doses above  $N_{s_0}$  were adjusted to a single linear model (Figure 2a). The model adjusted in N<sub>zero</sub> was consequence of N deficiency that affecting leaf life span and leaf emission rate, limits LAI expansion and determine stabilization of HM at lower values. After this stabilization of HM flowering induction occurred, also contributing to maintain HM constant instead stem elongation, because no more leaves are produced and because the rate of emission of new vegetative tillers is very low. In the regrowth period, treatment  $N_{50}$  also fitted to a linear model plateau (Figure 2b). The reason for this type of response was the induction of reproductive period, as occurred previously in the treatment N<sub>zero</sub>. In this same period, HM from treatments  $N_{100}$  and  $N_{200}$  were fitted with to linear and linear-plateau models, respectively (Figure 2b). The linear-plateau in  $N_{200}$  is an indication that, at this level of nitrogen fertilizer, grazing needs to be done at lower levels of LI than the programmed 95%. Instead of N doses, the RUE values during the establishment phase were lower than the value indicated by the general model proposed by Varlet Grancher et al. (1989). This low efficiency can be attributed to the temperatures below average (1<sup>st</sup> ten days of June <  $4.9^{\circ}$ C; 3<sup>rd</sup> ten days of July  $< 3.1^{\circ}$ C). In the regrowth phase RUE was affected mainly by N deficiency, except in N<sub>200</sub> as demonstrated in Figure 1. This deficiency restricts the development of an adequate leaf area for capturing PAR<sub>inc</sub> and decreases the RUE.



Figure 1. Dilution of N in plant tissue of annual ryegrass under different rates of N fertilization and model %N = 4.8(HM)<sup>-0.32</sup> (Lemaire and Sallete, 1984)



Figure 2. Model of accumulation of herbage mass (HM, g m<sup>-2</sup>) in function to the accumulation of absorbed photosynthetically active radiation (PAR<sub>a</sub>, MJ m<sup>-2</sup>) for the establishment (a) and regrowth (b) period of annual ryegrass under different rates of N fertilization

#### Conclusions

The accumulation of herbage mass of annual ryegrass as a function of PAR<sub>a</sub> fits to different models depending on the phase of the cycle and N availability. During establishment there is no response to N application higher than 100 kg ha<sup>-1</sup>, while in regrowths, the potential herbage mass accumulation is reached only with 200 kg ha<sup>-1</sup> of N. There is a need to verify the effects of split-N fertilization along the cycle of development of the culture and to investigate a more accurate time of forage removal.

#### References

Lemaire G. and Chapman D. (1996) Tissue flows in grazed plant communities. In: Hodgson J. and Illius A.W. (eds.) *The ecology and management of grazing systems*, CABI, Wallingford, UK, pp. 3–36.

Lemaire G. and Salette J. (1984) Relation entre dynamique de croissance et dynamique de prélèvement d'azote pour un peuplement de graminées fourragères: I. Étude de l'effet du milieu. *Agronomie* 4, 423–430. Monteith J.L. (1972) Solar radiation and productivity in tropical ecosystems. *Journal of Applied Ecology* 9, 747–766. Thornley J.H.M. and Johnson I.R. (2000) *Plant and crop modelling: a mathematical approach to plant and crop physiology*, The Blackburn Pres, Caldwell, NJ, 669 pp.

Varlet-Grancher C., Gosse G., Chartier M., Sinoquet H., Bonhomme R. and Allirand J.M. (1989) Mise au point: rayonnement solaire absorbé ou intercepté par un couvert végétal. *Agronomie* 9, 19–439.

## Production of different forage leguminous shrubs growth in the Canary Islands

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## Abstract

Four species of leguminous shrubs endemic to the Canary Islands (Spain) – *Chamaecytisus palmensis* (tagasaste), *Teline canariensis*, *Teline osyrioides sericea* and *Teline osyrioides osyrioides* – were evaluated from 1999 to 2008 as a source of forage. Plant survival, edible green matter / inedible matter fraction (EGM/IM) and production of edible dry matter (EDM) were evaluated at three cutting heights (30, 50, 70 cm above ground level). Samples were taken annually during these ten years. Significant statistical differences related to EDM production (P < 0.05) were observed among all species, except between *T. o. osyrioides* and *T. canariensis*. The highest production was found in *C. palmensis*: 2.28, 4.64, 4.02 Mg EDM ha<sup>-1</sup> per year for each cutting height (30, 50 and 70 cm, respectively). EGM/IM ratio showed significant lower values for *C. palmensis* (1.50) related to the three *Teline* species (from 2.98 to 3.25).

Keywords: forage production, Chamaecytisus palmensis, Teline species

#### Introduction

Leguminous shrub species have become of interest in animal production systems mainly for their potential use as a source of forage. Moreover, these shrubs can also contribute with different benefits in other areas, preventing problems such as soil erosion associated with fertility and production decrease, soil structure, organic matter and nutrients loss and reduction of water retention capacity. The Canarian Foundation for Reforestation (Foresta) warns that the Canary Islands is one of the regions of the world with the highest danger of desertification, with more than 90% of the Archipelago surface considered to be at high risk (Europa Press, 2009). Endemic leguminous forage shrub species from the islands are able to grow and develop in areas with low fertility and scarce precipitation. They also show high adaptation to the climatic conditions of our islands as they are native plants. The objective of the work is to study forage production of four leguminous shrub species endemic to the Canary Islands and to evaluate cultivation techniques for year-round forage supply.

## Materials and methods

The experimental plot was situated in La Laguna (Tenerife, Canary Islands, Spain) at 28°28'N, 16°19'W and 549 m a.s.l. The average annual rainfall was 508 mm and the average air temperature was 17°C. The soils were Ultic Haplustafs; clayish, fertile and rich in organic matter. No fertilizers were used on the plot during the experiment. The seeds were germinated in a chamber at a constant temperature of 16°C, with 16 h of light and 8 h of darkness. Forestry containers were used for sowing. The plantlets were grown for 5 months in a greenhouse and then transplanted to the field in February 1999. The field experiment had a split-plot design comprising main plots of four species (*Chamaecytisus palmensis* (tagasaste), *Teline canariensis, Teline osyrioides* 

*osyrioides* and *Teline osyrioides sericea*) and sub-plots of three cutting heights with four replicates of each plot arranged in a completely random design. Each sub-plot contained three rows of seven plants; each row was cut to a different plant height: 30, 50, and 70 cm above ground level. Rows were separated by a distance of 1.87 m while within rows plants were placed at intervals of 1.04 m (5142 shrubs ha<sup>-1</sup>).

The cuts were made when growing tips were between 35 and 80 cm long. A total of seventeen cuts were performed over the ten years (three in 1999, four in 2000, one in 2001, 2004, 2005, 2006, 2007 and 2008, and two 2002 and 2003). The first cut (spring 1999) was performed only on *C. palmensis*, which was the unique species that grew more than 100 cm of plant height. Total biomass production for each cutting height was weighed fresh. A sample of approximately 2 kg was taken from each plot for separation by hand to weigh the edible green matter (EGM) *versus* inedible matter (IM) (wood) fraction. About 500 g of the EGM fraction were dried at 60°C for 48 hours, to determine the content per g kg<sup>-1</sup> of edible dry matter (EDM content).

Statistical studies consisted of repeated measures ANOVA followed by LSD tests, and the nonparametric test of Kolmogorov-Smirnov to test the survival of plants, in relation to the plant species and the cutting height.

#### **Results and discussion**

Plant survival: the comparison of plant survival at different cutting heights does not show significant differences (P > 0.05) (Table 1), so we can conclude that cutting height has little influence on plant survival. In contrast, the species type notably determines plant survival. Survival of C. palmensis is clearly higher that survival of other species like T. o. osyrioides and T. canariensis. Main causes of loss were natural death and rabbit attack. Edible dry matter production: species show notable differences among them (Table 2). Chamae*cytisus palmensis* reached the highest mean EDM value: 3.65 Mg EDM ha<sup>-1</sup> year<sup>-1</sup>. The lowest values were obtained in T. o. osyrioides and T. canariensis with mean values of 1.30 and 1.65 Mg EDM ha<sup>-1</sup> year<sup>-1</sup>, respectively. Cutting height at 30 cm was in general the less productive (mean 1.69 Mg EDM ha<sup>-1</sup> year<sup>-1</sup>, Table 2). However, species factor interacts significantly (P < 0.05, not shown in Table 2) with cutting height factor; therefore 30 cm is the most productive and the recommended cutting height for T. canariensis. Cutting heights at 50 cm is recommended for C. palmensis, whereas a 70 cm cutting height is the best choice for T. o. sericea and T. o. osyrioides (Table 2). The maximum EDM production mean value, across all the experiment, was obtained in C. palmensis species at cutting heights of 50 cm (4.64 Mg EDM ha<sup>-1</sup> year<sup>-1</sup>).

Survival	Ζ	Р
According cutting height		
30 vs. 50	1.20	0.112
30 vs. 70	1.02	0.240
50 vs. 70	0.51	0.954
According species		
C. palmensis vs. T. canariensis	1.59	0.013
C. palmensis vs. T. o. osyrioides	2.12	0.000
C. palmensis vs. T. o. sericea	1.41	0.037
T. canariensis vs. T. o. osyrioides	1.06	0.211
T. canariensis vs. T. o. sericea	1.59	0.013
T. o. osyrioides vs. T. o. sericea	1.77	0.004

Table 1. Survival test (Z of Kolmogorov-Smirnov). Cutting heights (30, 50 and 70 cm).  $P \le 0.05$  has been considered significant

species sective in 199	2000. mean v		or or the mean) nor	ii the rotal repetitions
	30 cm	50 cm	70 cm	Mean
C. palmensis	2.28±0.33	4.64±0.66	4.02±0.50	3.65±0.31 c
T. canariensis	$1.74\pm0.19$	$1.62 \pm 0.11$	$1.60\pm0.12$	1.65±0.08 ab
T. o. osyrioides	$1.08 \pm 0.10$	1.33±0.11	$1.49 \pm 0.12$	1.30±0.06 a
T. o. sericea	1.67±0.23	2.40±0.20	2.61±0.33	2.23±0.15 b
Mean	1.69±0.12 a	2.50±0.20 b	2.43±0.17 b	

Table 2. Mean edible dry matter production (Mg EDM  $ha^{-1}$  year<sup>-1</sup>) at three cutting heights for four shrub species between 1999 and 2008. Mean value (± standard error of the mean) from the four repetitions

Table 3. Edible dry matter content (g kg<sup>-1</sup>) and edible green matter vs. inedible matter (wood) (EGM/IM) fraction in four shrub species. Mean value ( $\pm$  standard error of the mean) from the four repetitions, n = number of measures

	EGM/IM	EDM content (g kg <sup>-1</sup> )
n	160	160
C. palmensis	1.50±0.13 a	377±10 a
T. canariensis	3.18±0.32 b	400±9ab
T. osyrioides osyrioides	2.98±0.28 b	411±8 b
T. osyrioides sericea	3.25±0.48 b	416±20c

Edible green matter/inedible matter: EGM/IM variable show significant lower values ( $P \le 0.05$ ) for *C. palmensis* (1.50±0.13) in relation to the three *Teline* species (between 2.98±0.28 and 3.25±0.48) (Table 3). Douglas *et al.* (1996) report a maximum EGM value of 0.4, and a resultant edible vs. inedible ratio of 0.67 for forage shrubs. Mean values are even higher for all the species studied in the present work when comparing with data obtained by other authors (Milthorpe and Dann, 1991).

Edible dry matter content: EDM content show differences between *C. palmensis* (mean value:  $377 \text{ g kg}^{-1}$ ) and the three *Teline* species (values between 400 and 416 g kg<sup>-1</sup> (Table 3). EDM obtained results for all the studied species are higher than those presented by Chinea *et al.* (2007).

## Conclusions

The results provide evidence of the great potential of these shrubs for the provision of healthy animal feed year-round.

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#### References

Chinea E., García-Ciudad A., Barquín E. and García-Criado B. (2007) Evaluation of endemic leguminous forage shurbs from the Canary Islands. 1 Germplasm characterisation and forage production. *New Zealand Journal of Agricultural Research* 50, 417–427.

Douglas G.B., Bulloch B.T. and Foote A.G. (1996) Cutting management of willows (*Salix* spp.) and leguminous shrubs for forage during summer. *New Zealand Journal of Agricultural Research* 39, 175–184. Europa Press (2009) *Foresta. Alerta del peligro de la desertización en Canarias*. Diario de Avisos. (5/06/09), 30 pp.

Milthorpe P.L. and Dann P.R. (1991) Production from tagasaste (*Chamaecytisus palmensis*) at four contrasting sites in New South Wales. *Australian Journal of Experimental Agriculture* 31, 639–644.

## Biomass production and forage quality in multispecies swards

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## Abstract

Multispecies mixtures of legumes and grasses offer potential advantages over the perennial ryegrass monocultures and binary white clover-perennial ryegrass mixtures usually sown in temperate pastures. These include greater productivity, increased resistance to unsown species invasion and improved forage quality. An experiment comprising eight experimental communities containing different proportions of birdsfoot trefoil (*Lotus corniculatus*), red clover (*Trifolium pratense*), a high-sugar perennial ryegrass (*Lolium perenne*) and timothy (*Phleum pratense*) was established and results are presented from three years of measurements.

Keywords: birdsfoot trefoil, perennial ryegrass, red clover, timothy, yield, forage quality

## Introduction

The importance of species diversity may be underestimated if only one of several ecosystem services is considered (Hector and Bagchi, 2007). Analysis of the true functional benefit of diversity therefore requires simultaneous measurement of many ecosystem processes in swards. These could include biomass productivity, resistance to invasion by unsown species, efficiency of use of environmental resources, soil structuring processes and the provision of forage of high nutritive quality. There is already clear evidence that multispecies mixtures of forage legumes and grasses offer yield advantages over the perennial ryegrass monocultures and binary white clover-perennial ryegrass mixtures usually sown in temperate pastures (Kirwan et al., 2007). This research highlighted the importance of species evenness (i.e. relative abundance) in producing positive diversity effects in agronomically relevant mixtures of four species. The aim of the current experiment was to analyse the effect of contrasting initial levels of species evenness in multispecies mixtures on a range of ecosystem processes. To achieve this, evenness in a group of four temperate forage species was manipulated to create eight mixtures: four near-monocultures (low evenness) and four near-centroids (high evenness). This paper discusses the development of the mixtures over time in terms of biomass production, unsown species presence and forage quality, but other processes were also measured.

## Materials and methods

32 field plots (6 m<sup>2</sup>) containing eight multispecies mixtures (comprising different sown proportions of perennial ryegrass (PRG) (high sugar variety cv. AberDart), timothy (cv. S48), red clover (cv. AberRuby) and birdsfoot trefoil (cv. Leo)) were established in 2008 in a randomised block design with four replicates (Table 1). The experiment was managed under an agronomically realistic conservation cutting regime, i.e. 3 cuts per year (late spring, mid-summer, late summer) at a height of 4 cm. The plots received a total of 90 kg N ha<sup>-1</sup> yr<sup>-1</sup> applied in three equal doses. Harvested biomass was subsampled and separated into its sown and unsown species components, oven-dried at 80°C and weighed. Representative sub-samples were also collected from each plot at each harvest, oven dried at 60°C and finely milled in preparation for standard chemical analyses of the major forage quality parameters. This

paper focuses on DOMD (digestible organic matter content) and relationships between concentrations of WSC (water soluble carbohydrate) and CP (crude protein). Results were analysed by ANOVA, focusing on species evenness effects on annual dry matter yield, and on mixture effects for forage quality. Results of sward yield are presented for 2009 (Year 1), 2010 (Year 2) and 2011 (Year 3). Forage quality results are presented for 2009 and 2010.

#### **Results and discussion**

The yield of both low and high evenness mixtures decreased sharply after Year 1 but remained stable in subsequent years (Table 2). High evenness mixtures were significantly more productive in all years. Over the three years of the experiment the yield of unsown species increased substantially (36, 297 and 1194 kg ha<sup>-1</sup> in Years 1, 2 and 3), but did not differ significantly between the evenness treatments, except in Year 2 in which it was lower in the high-evenness mixtures. Forage quality results are presented in Table 3.

Table 1. Composition of mixtures presented as proportions of the standard monoculture sowing rate for each species. Standard monoculture sowing rates used were: lotus = 10 kg ha<sup>-1</sup>; red clover = 10 kg ha<sup>-1</sup>; PRG = 20 kg ha<sup>-1</sup>; timothy = 20 kg ha<sup>-1</sup>

0 ,	0	<i>,</i> ,	0		
Mixture	Evenness	Lotus	Red clover	PRG	Timothy
1	Low	0.91	0.03	0.03	0.03
2	Low	0.03	0.91	0.03	0.03
3	Low	0.03	0.03	0.91	0.03
4	Low	0.03	0.03	0.03	0.91
5	High	0.34	0.22	0.22	0.22
6	High	0.22	0.34	0.22	0.22
7	High	0.22	0.22	0.34	0.22
8	High	0.22	0.22	0.22	0.34

Table 2. Annual sow	n species	yield (k	ig ha-1)
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Evenness	Year 1	Year 2	Year 3
Low	10869	5397	6144
High	14235	6582	7085
F prob.	0.002	0.046	0.03

In Year 1 there was no significant effect of mixture on DOMD at any harvest. The overall mean values in cuts 1, 2 and 3 were 59.38%, 63.71% and 63.93% respectively. There were significant differences between mixtures for herbage CP in all harvests in Year 1. Highest levels were found in mixtures containing a high proportion of legumes (1 and 2), whilst mixtures dominated by grasses (3 and 4) contained the lowest amounts. There were significant differences between mixtures in herbage WSC in all harvests. This parameter was strongly influenced by the presence of the high sugar PRG cv. AberDart, and was therefore highest in mixture 3. The second grass in the experiment, timothy, was chosen to provide a functional contrast to the high sugar PRG, as this species is naturally relatively low in WSC. This contrast was evident in the results, in which lower WSC levels were observed in mixture 4 (dominated by timothy). In Year 2 the mixtures did not differ in DOMD in cut 1 and the overall mean was 64.8%. In cut 2 there was a significant mixture effect, with the highest value (66.9%) found in the Lotus near-monoculture (mixture1), and the lowest (63.8%) in the timothy near-monoculture (mixture 4). DOMD values were substantially lower in all mixtures in cut 3 and there was a significant mixture effect. The highest value (62.6%) was in mixture 8 and the lowest in mixture 4 (58.5%). Overall, the DOMD values

reported here are low: expected average values for a monoculture of PRG cv. AberDart in Aberystwyth would be about 71%. The ratio between WSC and CP in forage is important in terms of ruminant nutrition. The ideal ratio has been calculated as around 2.4:1, but this was not achieved by any treatment in either Year 1 or Year 2. In both years the highest ratios were observed in mixture 3 in cut 1, showing the benefit of the high sugar trait. However, all other mixtures and cuts were deficient in WSC due to the low yield of the high sugar PRG relative to the high yield of timothy (results not shown).

Forage quality				Mix	ture				F prob.
Cut 1	1	2	3	4	5	6	7	8	
DOMD	58.82	59.07	61.03	56.85	60.21	58.05	58.69	59.38	0.284
WSC	8.58	6.65	13.59	6.91	6.55	6.86	6.64	6.36	< 0.001
СР	9.82	12.2	9.51	9.24	11.86	11.11	11.26	12.43	0.002
Cut 2									
DOMD	63.3	63.57	64.76	63.98	63.7	64.4	63.9	62.07	0.086
WSC	4.16	5.13	8.99	6.92	5.34	5.74	5.72	4.99	< 0.001
CP	16.91	16.21	14.1	14.76	16.58	16.12	16.0	16.12	0.002
Cut 3									
DOMD	62.62	67.32	64.7	58.96	66.37	65.68	66.06	63.93	0.072
WSC	9.44	7.03	13.07	10.64	9.38	9.22	9.89	10.75	< 0.001
СР	16.69	18.54	13.91	13.31	16.58	17.64	16.18	16.09	< 0.001
Year 2									
Forage quality				Mix	ture				F prob.
Cut 1	1	2	3	4	5	6	7	8	
DOMD	66.17	64.39	65.85	64.41	64.22	64.37	64.67	64.52	0.236
WSC	7.78	9.15	13.31	10.24	14.29	11.14	13.69	13.37	0.030
CP	16.52	13.67	12.33	13.67	12.44	12.62	11.94	11.66	< 0.001
Cut 2									
DOMD	66.92	64.43	64.58	63.85	65.53	64.61	65.31	65.51	0.020
WSC	7.25	7.0	9.07	8.27	7.77	7.4	7.89	8.35	0.176
CP	18.59	15.8	14.8	15.34	16.44	15.72	16.16	15.94	0.017
Cut 3									
DOMD	60.78	61.27	58.57	58.56	61.06	60.75	59.5	62.64	0.002
WSC	6.59	7.5	9.09	8.03	8.72	7.99	8.46	7.26	0.007
СР	15.59	15.02	12.91	13.23	14.38	14.23	13.77	15.33	0.016

Table 3. Forage quality parameters (% DM) for each mixture Year 1

In conclusion, there were distinct yield gains in high evenness mixtures in terms of sown species, but increased resistance to unsown species invasion was not a stable characteristic of these mixtures. The nutritive value of harvested forage did not differ markedly between the two levels of species evenness.

#### Acknowledgements

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#### References

Hector A. and Bagchi R. (2007) Biodiversity and ecosystem multifunctionality. *Nature* 448, 188–190. Kirwan L. *et al.* (2007) Evenness drives consistent diversity effects in an intensive grassland system across 28 European sites. *Journal of Ecology* 95, 530–539.

## Effect of grass species and ploidy on clover content in grass-clover mixtures

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#### Abstract

Tall fescue (*Festuca arundinacea* Schreb.) is of increasing interest in Western Europe, mainly because of its good drought resistance. Agronomic data on this species are relatively scarce and little is known about the compatibility of tall fescue with clover. We established field trials in 2009 to compare the yield, botanical composition, feeding value and N-fixation of different grass species associated with clover. A first trial compared tall fescue, diploid perennial ryegrass (*Lolium perenne* L.) and tetraploid perennial ryegrass sown with white clover (*Trifolium repens* L.). A second trial compared tall fescue, diploid Italian ryegrass (*Lolium multiflorum* Lam.) and tetraploid Italian ryegrass sown with red clover (*Trifolium pratense* L.). In all plots 5 cuts were taken in both 2010 and 2011and the yield, botanical composition, nitrogen content and digestibility of the harvested material were determined. Preliminary conclusions show no effect of grass species on white clover content in the mixtures. Red clover content in turn was consistently higher in combination with tall fescue than with Italian ryegrass.

Keywords: tall fescue, red clover, white clover, perennial ryegrass, Italian ryegrass, yield

#### Introduction

The grass species tall fescue (*Festuca arundinacea* Schreb.; Fa) is of increasing interest in Western Europe. Contributing factors are the expectation of dryer summers due to global climate change and trends towards more cutting and less grazing in dairy farming. So far, most of the grass-clover research has focused on perennial ryegrass (*Lolium perenne* L.; Lp) and Italian ryegrass (*Lolium multiflorum* Lam.; Lm) in combination with white clover (*Trifolium repens* L.; Tr) and/or red clover (*Trifolium pratense* L.; Tp). Little is known about the compatibility of Fa with white/red clover. Annicchiarico and Proietti (2010) found that under North-Italian conditions Fa allowed the highest yield and highest clover content compared to other grass species.

We compared Lp and Fa in combination with Tr and Lm, and Fa in combination with Tp. Since Fa has a more open growth habit than Lp, we expected that white clover content would be lower with the latter. In analogy, we expected a higher clover content with tetraploid ryegrass than with diploid ryegrass due to the more open growth habit of the former. Growth of Lm is very high in spring and weak in summer, whereas the growth of Fa is more regular through the season. Hence our third hypothesis was that red clover content would fluctuate less in combination with Fa compared to Lm.

#### Materials and methods

The first trial, comparing Fa (cv. Castagne), diploid Lp (Lp2, cv. Plenty) or tetraploid Lp (Lp4, cv. Roy) with Tr (cv. Merwi), was sown in April 2009. The second trial, comparing Fa, diploid Lm (Lm2, cv. Melclips) or tetraploid Lm (Lm4, cv. Melquatro) with Tp (cv. Merviot), was sown in September 2009. Both trials were established on a sandy loam soil in Merelbeke (Flanders, Belgium) and were designed as randomized complete blocks

with three replicates. Individual plot size was 7.8 m<sup>2</sup>. Grass was sown at a density of 1000 viable seeds m<sup>-2</sup>, Tr at a density of 700 viable seeds m<sup>-2</sup> and Tp at a density of 600 viable seeds m<sup>-2</sup>. Annual fertilisation amounted to 150 kg N ha<sup>-1</sup> 26 kg P ha<sup>-1</sup> and 266 kg K ha<sup>-1</sup>. Five cuts were harvested each year. Subsamples were taken for separation into the constituent species and for chemical analyses. Digestibility and crude protein content were determined using NIRS. On the moment of the submission of this paper, digestibility and crude protein data were not yet available for 2011. Statistics were performed using the Anova function in R (R development core team, 2011) with block as random factor, and grass species as fixed factor. Multiple comparison was done using the TukeyHSD function.

#### **Results and discussion**

Fa+Tr outyielded Lp+Tr by more than 20% in 2010 and by more than 23% in 2011. This difference was significant in 2011 (P < 0.001). The organic matter digestibility (OMD), on the other hand, was 6 units lower for Fa+Tr compared to Lp+Tr (P < 0.001) in 2010. In 2010 the clover contents in the total DM yields of Lp4+Tr and Fa+Tr were, as we expected, higher than these in the Lp2+Tr mixture, but this was not the case in 2011. Differences in white clover content between the species or between the diploid and tetraploid Lp were not significant (Table 1). Our results are in line with Frame and Boyd (1986) and Elgersma and Schlepers (1997) who observed, respectively, only minor or no differences in clover content between diploid and tetraploid perennial ryegrass. Mean white clover content in the total dry matter (DM) yield was 20.7% in 2010 and 23.4% in 2011. These values are comparable to those found by Williams et al. (2003) at similar N fertilization. In both years, white clover content in the sward increased towards the end of the season (Figure 1a). In the 3<sup>rd</sup> cut of 2010, there was a sudden increase in the clover proportion in Lp+Tr but not in Fa+Tr. We expect this to be due to a severe period of drought which hampered the growth of Lp more than the growth of Fa. Crude protein content in the mixtures in 2010 was not significantly different, but the higher yield of Fa+Tr resulted in a significantly higher nitrogen (N) yield (P < 0.05) of Fa+Tr compared to Lp+Tr in 2010. Since the clover content was similar in both mixtures, we presume the grass component to be responsible for the N-difference: i.e. the deeper rooting system of Fa might have intercepted more N than that of Lp.

				2011			
	DMY	CC	OMD(0/)	СР	NY	DMY	CC
	(Mg ha <sup>-1</sup> )	(%)	OMD (%)	(%)	(kg ha <sup>-1</sup> )	(Mg ha <sup>-1</sup> )	(%)
Fa+Tr	14.7ª	20.7ª	75.3 <sup>b</sup>	15.7ª	368.0ª	16.0ª	22.5ª
Lp2+Tr	12.2ª	18.8ª	81.1ª	14.8 <sup>a</sup>	288.8 <sup>b</sup>	12.4 <sup>b</sup>	24.2ª
Lp4+Tr	11.6 <sup>a</sup>	22.5ª	81.3ª	15.9ª	294.8 <sup>b</sup>	13.0 <sup>b</sup>	23.5ª
Fa+Tp	15.9ª	52.9ª	73.5 <sup>b</sup>	19.5ª	496.2ª	19.5ª	49.4ª
Lm2+Tp	18.7ª	25.9 <sup>b</sup>	77.2ª	14.6 <sup>b</sup>	438.6ª	19.6ª	40.5 <sup>a,b</sup>
Lm4+Tp	17.8 <sup>a</sup>	24.2 <sup>b</sup>	77.8 <sup>a</sup>	15.5 <sup>b</sup>	440.4ª	19.2ª	39.5 <sup>b</sup>

Table 1. Mean annual Dry matter yield (DMY), clover content in DMY (CC), organic matter digestibility (OMD), crude protein content (CP) and nitrogen yield (NY) of grass clover mixtures. Treatments with the same letter in the same column are not significantly different (Tukey test, P < 0.05)

Fa: *Festuca arundinacea*, Lp: *Lolium perenne*, Tr: *Trifolium repens*, Lm: *Lolium multiflorum*, Tp: *Trifolium pratense*, Lp2: diploid Lp, Lp4: tetraploid Lp, Lm2: diploid Lm, Lm4: tetraploid Lm.

Lm+Tp overyielded Fa+Tp by approximately 15% in 2010. In 2011 yields of both species with red clover were similar. Difference in yield in the first year after (autumn) sowing can be attributed to the slow establishment rate of Fa compared to Lm. Mean red clover content

in the total DM yield was 34.3% in 2010 and 43.1% in 2011. Red clover content in total DM yield was twice as high in Fa+Tp compared to Lm+Tp in 2010. In 2011 the clover content was again higher with Fa compared to Lm, but the difference was smaller. There was no difference in red clover content between the diploid and the tetraploid Italian ryegrass. As we expected, red clover content within a year was more constant in Fa+Tp compared to Lm+Tp (Figure 1b).The OMD of Fa+Tp was significantly lower than that of Lm+Tp (P < 0.001) in 2010 (Table 1). As a result of the higher clover content of Fa+Tp, both the CP content and the N yield of Fa+Tp were higher compared to Lm+Tp in 2010 (Table 1). This difference was significant for CP content (P < 0.001).



Figure 1. (a) Clover content in the DM yield in mixtures of white clover (Tr) with tall fescue (Fa), diploid perennial ryegrass (Lp2) and tetraploid perennial ryegrass (Lp4)) over 10 consecutive cuts in 2 years. b) Clover content in mixtures of red clover (Tp) with tall fescue (Fa), diploid Italian ryegrass (Lm2) and tetraploid Italian ryegrass (Lm4) over 10 consecutive cuts in 2 years

#### Conclusions

We found no significant difference in clover content in swards where either tall fescue or perennial ryegrass were combined with white clover, during neither of the first two years after the sowing year. In both years, red clover content was significantly higher and fluctuated less in combination with tall fescue compared to Italian ryegrass. We found no significant effect of the ploidy of the perennial or Italian ryegrass, nor on either the red or on the white clover.

## References

Annicchiarico P. and Proietti S. (2010) White clover selected for enhanced competitive ability widens the compatibility with grasses and favours the optimization of legume contents and forage yields in mown clover-grass mixtures. *Grass and Forage Science* 65, 318–324.

Elgersma A. and Schlepers H. (1997) Performance of white clover/perennial ryegrass mixtures under cutting. *Grass and Forage Science* 52, 134–146.

Frame J. and Boyd A. (1986) Effect of cultivar and seed rate of perennial ryegrass and strategic fertilizer nitrogen on the productivity of grass/white clover swards. *Grass and Forage Science* 41, 359–366.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Williams T., Evans D., Rhodes I. and Abberton M. (2003) Long-term performance of white clover varieties grown with perennial ryegrass under rotational grazing by sheep with different nitrogen application. *Journal of Agricultural Science Cambridge* 140, 151–159.

# Effect of water saturation of soil on winter survival of red clover (*Trifolium pratense*) and timothy (*Phleum pratense*) in Norway

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## Abstract

Climate change is predicted to cause water saturation of soil in some Nordic regions due to increased precipitation in autumn. An outdoor pot experiment was performed at two sites in Norway (Fureneset (69°39'N) and Holt (59°45'N)) to examine whether such soil saturation affects hardening of red clover (*Trifolium pratense*) and timothy (*Phleum pratense*). Two populations of red clover, adapted to either the southern or northern Nordic climate, and a Norwegian cultivar of timothy, were treated with two levels of soil water saturation (25%, 90%) during two periods of different lengths within the hardening process. The temperature at which 50% of plants were killed ( $LT_{50}$ ) was determined during autumn and winter to estimate the frost tolerance of the plants after hardening. Water-logged soil enhanced frost tolerance at colder temperatures (Holt), but had no effect at higher temperatures (Fureneset). Timothy had the lowest  $LT_{50}$  value, while the northern adapted clover had a lower  $LT_{50}$  value than the southern type. In spring there were no surviving clover plants at Holt, but at Fureneset the northern adapted clover with shorter water treatment had the best survival. Increased abiotic stress caused by higher levels of water saturation in soil may enhance frost tolerance at low temperatures, but may reduce it at higher temperatures because of higher respiration and reduced assimilation rates and thus reduced hardening of red clover.

Keywords: red clover, water-logging, anoxia, hardening, frost tolerance, climate change

## Introduction

Future climate change scenarios for Norway indicate increased precipitation during autumn (Reg-Clim, 2005). This can lead to water saturation of soils, causing anaerobic conditions and oxygen deficiency within the root. Anaerobic respiration results in a decrease in ATP production and energy shortage in the plant, and prolonged energy shortage may reduce winter survival of the plant (Licausi, 2010). Timothy (*Phleum pratense*) has been reported to be more resistant and to maintain higher carbohydrate reserves than red clover (*Trifolium pratense*) under oxygen deficiency (Bertrand *et al.*, 2003). The winter hardening of plants takes place at low positive temperatures (°C) as days grow shorter during autumn. In this experiment we investigated whether waterlogging of soil in autumn affected the hardening of two populations of red clover and timothy. Timothy is the most commonly grown forage species in Norway, but red clover may become increasingly important in the future as a forage species, generally as an important protein source and nitrogen supplier to the soil. Today, it is at its geographical limit for winter survival in northern Norway and has unacceptably low persistency in meadows in southern Norway.

## Materials and methods

The experiment was carried out at two sites in Norway: Fureneset, Fjaler (59°45'N) and Holt, Tromsø (69°39'N). The plant material used comprised two populations of red clover, adapted

to either a southern climate (S-pop; origin Czech Republic) or a northern climate (N-pop; origin northern Norway) relative to Nordic conditions, and one commercial timothy cultivar of South Norwegian origin (cv. Grindstad). Seedlings were established in the greenhouse and transferred to 10  $dm^3$  pots (pot height: 22.8 cm), with 10 plants per pot. The growth medium was 3:2 (vol/vol) peat: fine sand. The pots were kept outside from summer 2010 to June 2011. Plants were fertilised according to demand. The water-logging treatment was applied by placing the pots in watertight boxes with a water height of either 3 cm (field capacity, intended to maintain approx. 25 vol.-% soil water saturation) or 20 cm (full water saturation, approx. 90 vol.-% soil water saturation). Due to different site latitudes, treatment started on 16 September at Holt and 27 September at Fureneset. At Holt, the pots were kept within the boxes with continuous water content until spring. At Fureneset, the pots received treatment for either one or two months. In order to measure the degree of hardening of the plants,  $LT_{50}$  tests were performed twice at each site for estimation of frost tolerance. At Holt, the first LT<sub>50</sub> test was performed after six weeks of treatment and the second after 10 weeks. At Fureneset, the first LT<sub>50</sub> test was performed after one month of treatment and the second in mid-winter (Table 1). The LT<sub>50</sub> tests were performed using computer-controlled freeze boxes in the laboratory according to Larsen (1978). In brief, roots were washed and crown segments, with about 1-2 cm root length and 3 cm shoot length, were covered with humid sand in plastic boxes and gradually frozen down to pre-determined temperatures. Unfrozen controls were kept at a constant temperature of 2°C. Ten plants per population were used for each temperature, including two replicates per temperature.  $LT_{50}$  was estimated by scoring the regrowth of the plants after 3-4 weeks at room temperature and carrying out probit analyses using the logistic distribution in PROC PROBIT (SAS, 2004). Feducial limits ( $\alpha = 0.05$ ) were used to test any significant differences between treatments. Two pots per treatment for each population/cultivar (a total of 24 pots at each site) were kept outside until spring to be scored for plant survival.

#### **Results and discussion**

The  $LT_{s_0}$  values for timothy and red clover are presented in Table 1. Timothy was more frost tolerant than red clover. At Fureneset there was no difference between timothy grown at field capacity or in fully saturated soil. At Holt, on the other hand, timothy in fully saturated soil had higher frost tolerance than timothy at field capacity. Studies from Poland also reported increased hardening of meadow fescue (Festuca pratensis) acclimated at higher water saturation level (Rapacz and Jurczyk, 2009). An earlier study in Norway showed no decrease in hardening of timothy as an effect of full soil water saturation when the temperature during autumn was high, i.e. water was needed for plant growth, whereas when autumn temperatures were lower,  $LT_{50}$  values was reduced for the most frost sensitive timothy cultivar investigated (Østrem et al., 2009). In our study, N-pop of red clover generally had higher frost tolerance than S-pop. In the first LT<sub>50</sub> test at Holt, the plants at field capacity were more frost tolerant, while in the second  $LT_{50}$  test at Holt and the first at Fureneset, the plants in fully water-saturated soil were more frost tolerant. In the second LT<sub>50</sub> test at Fureneset the plants at field capacity were more frost tolerant. Red clover has a different energy storage mechanism than timothy in that it stores carbohydrates in the form of starch instead of fructans. Studies in Canada showed that total non-structural carbohydrates (TNC) increased in red clover during autumn, reached a maximum in November and then decreased during the overwintering period, with a faster rate of decrease under oxygen deficiency. In timothy, on the other hand, TNC increased progressively from autumn to February, with a higher increase under oxygen deficiency (Bertrand et al., 2003). This can partly explain the different results obtained for red clover and timothy. In addition to climate differences, the differences observed here between Holt and Fureneset may have been caused by growth stage of the plants, with larger plants at Fureneset.

			]	Fureneset			Holt			
	Degree of	IТ	1) 27	Oct. 2) 19	) Jan.	1) 29	1) 29 Oct. 2) 23			
	satura-	LI 50		95% Co	nfidence		95% Co	nfidence		
	tion (%)	lest	LT 50	inte	rval	$LT_{50}$	inte	rval		
			50	lower	upper	50	lower	upper		
	25	1	-17.0	-17.7	-16.2	-17.3	-18.0	-16.6		
Timothy	90	1	-16.5	-17.3	-15.8	-19.2	-19.9	-18.5		
(cv. Grindstad)	25	2	-23.9	-25.0	-22.7	-17.1	-18.3	-16.0		
	90	2	-23.2	-24.3	-22.1	-22.6	-23.9	-21.5		
Dad alayan	25	1	-10.6	-11.4	-9.8	-12.5	-13.3	-11.8		
(Naran aniain Narah	90	1	-11.7	-12.5	-11.0	-10.9	-11.7	-10.2		
(N-pop, origin North-	25	2	-16.5	-17.6	-15.5	-13.3	-14.5	-12.2		
ern Norway)	90	2	-12.6	-13.5	-11.7	-15.4	-16.7	-14.3		
Ped clover	25	1	-9.5	-10.3	-8.6	-9.1	-9.9	-8.3		
(S non ori	90	1	-10.3	-11.1	-9.5	-7.0	-7.7	-6.2		
(S-pop, off-	25	2	-9.9	-10.9	-9.0	-6.0	-7.3	-4.7		
gin Czech Republic)	90	2	-8.7	-9.8	-7.5	-12.0	-13.4	-11.0		

Table 1. Frost tolerance as  $LT_{50}$  values and 95% confidence interval for timothy and red clover at two levels of soil water saturation at two sites in Norway (Fureneset, Holt). Dates of the two  $LT_{50}$  tests (1, 2) are also indicated

#### Conclusions

Full water-saturation of soil in autumn enhanced the frost tolerance of timothy at colder temperatures (Holt), but had no effect at higher temperatures (Fureneset). Red clover adapted to the northern Norwegian climate (N-pop) generally showed higher frost tolerance than a red clover population adapted to the southern Norwegian climate (S-pop).

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#### References

Bertrand A., Castonguay Y., Nadeau P., Laberge S., Michaud R., Bélanger G. and Rochette P. (2003) Oxygen deficiency affects carbohydrate reserves in overwintering forage crops. *Journal of Experimental Botany* 54, 1721–1730.

Larsen A. (1978) Freezing tolerance in grasses. Methods for testing in controlled environments. *Meld. Norges Landbrukshøgskole* 57, 56 pp.

Licausi F. (2010) Regulation of the molecular response to oxygen limitations in plants (Minireview). *New Phytologist* 190, 550–555.

Rapacz M. and Jurczyk B. (2009). Environmental regulation of cold acclimation in herbaceous plants – physiological and molecular response. *PMAC 2009*, December 4–8,2009, Aas, Norway, p. 24.

RegClim (2005) Norges klima om 100 år. Usikkerhet og økt risiko. (In Norwegian). http://regclim.met.no. Østrem L., Höglind M. and Jørgensen M. (2009) Vinterbiologi i endra klima – vassoverskot (in Norwegian). *Bioforsk conference*, Oslo 2009.

SAS (2004) SAS Online Doc® 9.1.3. Cary, NC, USA: SAS Institute Inc.

# The effect of establishment method and seed mixture composition on dock (*Rumex crispus* L.) establishment

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## Abstract

A field trial with the aim to evaluate establishment methods and seed mixture was established in 2009–2010. Seeds of *Rumex crispus* L. were sown into all plots before the establishment of the ley crop. The ley crop was harvested three times in 2011. Plant numbers of *R. crispus* were counted in a sub-plot in spring and in the autumn of 2011, and visually scored before each harvest. The number of established *R. crispus* plants was reduced by up to 50% in treatments where the ley was undersown in barley grown to maturity, compared to establishment methods where the nurse crop did not provide a full cover over the summer.

Keywords: Rumex spp., establishment method

## Introduction

*Rumex* spp. are troublesome grassland weeds common to all European countries (Zaller, 2004). They are difficult to control once they are established, and they form a persistent seed bank in grasslands from which seedlings may establish even if all mature plants are eradicated. However, seedlings are vulnerable to competition during the establishment phase (Bond *et al.*, 2007). In Sweden, *Rumex* spp. are increasingly perceived as a problem in short-term leys, particularly under organic management. The objective of this study was to evaluate the effect of different establishment methods and seed mixtures on the establishment of *Rumex crispus* L. from the soil seed bank.

## Materials and methods

A field trial was established at Rådde (57°36' N, 13°15' E) in the autumn of 2009, after treatment with RoundUp (28 August) and ploughing. The soil texture at the site is dominated by sand and silt (88%), pH was 6.0, and the content of soluble phosphorus and potassium were 11.3 and 5.3 mg  $100g^{-1}$  soil, respectively. The site was not affected by *Rumex* spp. Seeds of *R*. crispus L. were collected in the autumn of 2008 and stored outdoors until the establishment of the experiment in late summer of 2009. Ten g Rumex seed (c. 3500 seeds with a germination rate of 75% as determined in a Petri dish test in a weakly lit chamber at fluctuating temperature  $(+5/+20^{\circ}C)$ ) was broadcast and raked into each plot  $(16 \text{ m}^2)$  in Sept. 2009. There were 10 treatments, specified in Table 1. The control was the most common establishment method and the most common seeding mixture treated with 15 g Gratil + 0.6 l MCPA ha<sup>-1</sup> after the emergence of the crop and some *Rumex* plants. Other treatments received no herbicide. Whole-crop barley silage (treatment D) was harvested on 14 July, mature barley (4.8 tons ha<sup>-1</sup>) and winter wheat (3.8 tons ha<sup>-1</sup>) were harvested on 31 August, and Lolium *multiflorum* was harvested on 14 July, 12 August and 22 September (6.3 tons dry matter ha<sup>-1</sup> in total). All plots received N, P and K according to recommended rates. In 2011 all plots were harvested at 9 June, 19 July and 14 September. R. crispus plant numbers were visually scored for abundance (0-10), where 0 = n0 plants and 10 = high abundance of plantsacross the whole plot) before each harvest in 2011. R. crispus plant numbers were counted in a 0.25 m<sup>2</sup> sub-plot at the centre of each plot in the spring and the autumn of 2011. The experimental layout was a randomized block design with four replicates. ANOVA analysis was carried out with Proc GLM in SAS.

Treatments	Nurse crop, seeding date	Seeding time, ley crop	Seeding mixture*
A, control	Barley, 6 May 2010	Same as nurse crop	Basic
В	Barley, 6 May 2010	Same as nurse crop	Basic
С	Barley, 6 May 2010	27 May 2010	Basic
D	Whole-crop barley, 6 May 2010	Same as nurse crop	Basic
E	L. multiflorum, 6 May 2010	Same as nurse crop	Basic
F	Barley, 6 May 2010	Same as nurse crop	Basic + L. perenne
G	Barley, 6 May 2010	Same as nurse crop	Basic + Festulolium
Н	Barley, 6 May 2010	Same as nurse crop	Basic + C. intybus
Ι	Fallow with repeated harrowings	5 July 2010	Basic
J	Winter wheat, 17 Sept. 2009	6 May 2010	Basic

Table 1. Establishment methods and seeding mixtures

\*Basic = T. pratense, T. repens, P. pratense, F. pratensis.

#### **Results and discussion**

There were few significant effects of establishment method and seeding mixture on the yield of the established ley crop. However, three treatments (*L. multiflorum*, under-sowing in winter wheat, and the late establishment of the ley after a short fallow) resulted in significantly poorer ley performances (Table 2). Significantly more *R. crispus* plants were established in the plots where the nurse crop was either *L. multiflorum* or whole-crop silage. The *R. crispus* plant numbers recorded in treatment E amounted to more than 50% of the seeds sown. It is interesting to note that herbicide treatment did not result in fewer established *R. crispus* plants compared to treatments without herbicide treatment.

Treatment	V	isual scorir	ng (0–10)	Plant	numbers	DM yield of ley
Treatment	9 June	19 July	12 September	6 May	4 October	kg ha <sup>-1</sup>
А	1.25 <sup>a</sup>	2.75ª	3.75ª	3.50 <sup>a</sup>	3.25 <sup>a</sup>	11339ª
В	1.75 <sup>a</sup>	2.75ª	3.75 <sup>a</sup>	2.00 <sup>a</sup>	$2.00^{a}$	$11478^{a}$
С	7.5 <sup>b</sup>	5.25 <sup>b</sup>	6.00 <sup>b</sup>	2.25ª	3.00 <sup>a</sup>	10182 <sup>b</sup>
D	6.25 <sup>b</sup>	4.75 <sup>b</sup>	5.75 <sup>b</sup>	6.25 <sup>bc</sup>	4.75 <sup>ab</sup>	11451ª
E	6.5 <sup>b</sup>	5.75 <sup>b</sup>	7.00 <sup>b</sup>	7.25°	7.25 <sup>b</sup>	8970°
F	1.25 <sup>a</sup>	$2.00^{a}$	$2.00^{a}$	3.75ª	2.50 <sup>a</sup>	11651ª
G	$1.00^{a}$	2.00 <sup>a</sup>	$1.00^{a}$	3.50 <sup>a</sup>	3.25 <sup>a</sup>	11983 <sup>a</sup>
Н	2.25ª	3.00 <sup>a</sup>	3.25 <sup>a</sup>	3.25ª	3.50 <sup>a</sup>	11566 <sup>a</sup>
Ι	$8.00^{b}$	4.50 <sup>b</sup>	7.25 <sup>b</sup>	3.50 <sup>a</sup>	3.75 <sup>a</sup>	9844 <sup>b</sup>
J	6.75 <sup>b</sup>	5.75 <sup>b</sup>	6.75 <sup>b</sup>	4.50 <sup>ac</sup>	3.75 <sup>a</sup>	10662ª

Table 2. Visual scoring and counted plant numbers of R. crispus and DM yield of the ley crop in 2011

Figures with the same letter within a column are not significantly different.

Visual scoring suggests that the treatment where the ley crop was seeded late in the season, or with winter wheat as nurse crop, also contained more *R. crispus* plants, but it could also be that *R. crispus* plants in these plots were larger and thus more visible. The results suggest that access to light during the establishment phase is of primary importance for the survival of *R. crispus* seedlings. Despite the fact that *L. multiflorum* is a competitive species which suppressed the establishment of the ley components, this treatment resulted in the highest number of established *R. crispus* plants. The short periods after each harvest with ample access to light appear to be sufficient for *R. crispus* plants to get established. This is consistent

with results by Hongo (1989). The addition of a competitive species such *Festulolium* or *L. perenne* to the less-competitive basic seed mixture did not reduce the establishment of *R. crispus*. The addition of *Cichorium intybus* to the seed mixture, which belongs to the same functional type as *R. crispus*, did not have any effect on *R. crispus* establishment. A final evaluation will be carried out in spring 2012.

#### Conclusions

It is concluded that a spring sown nurse crop with traditional crop harvest late in the season decreased the number of *R. crispus* plants established from the soil seed bank. This treatment also resulted in the best establishment of the ley crop.

#### References

Bond W., Davies G. and Turner R.J. (2007) The biology and non-chemical control of broad-leaved dock (*Rumex obtusifolius* L.) and curled dock (*R. crispus* L.). Accessed at http://www.gardenorganic.org.uk/ organicweeds.

Hongo A. (1989) Survival and growth of seedlings of *Rumex obtusifolius* L. and *Rumex crispus* L. in newly sown grassland. *Weed Research* 29, 7–12.

Zaller J.G. (2004) Ecology and non-chemical control of *Rumex crispus* and *R. obtusifolius* (Polygonaceae): a review. *Weed Research* 44, 414–432.

# Effect of alternating hay/pasture utilization on the productivity of a legume-grass ley on an organic farm

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## Abstract

The aim of field experiments, carried out in 2009–2012 on an organic farm operated by PIB IUNG at Grabów (Poland, Masovian voivodeship, 51°21' N, 21°40' E), was to evaluate the effect of hay vs. pasture management on the yield and utilization of a short term legume-grass ley. In a field experiment laid out as a split-block design it was found that the alternating hay/pasture management reduced productivity. The highest and the most consistent yield and dry matter yields were obtained from alfalfa-grass swards and from white clover and alfalfa with grasses.

Keywords: organic farming, legume-grass mixtures, alternating hay/pasture utilization, pasture, sward productivity

## Introduction

Previous studies have shown that the method of defoliation affects the productivity and species composition of swards. The productivity of a legume-grass sward under grazing management has been reported to be usually inferior to the output of hay swards (Gaweł, 2000). Results in the literature have also shown a lower stability of mixtures caused by loss of legume plants and thinning of the sward as a result of the grasses being lost from the stand under prolonged grazing (Harasim, 2004). The author concluded that the alternating of pasture with hay contributed to an increase in the annual yield, in intake by cows and in the density of the white clover-grass sward (Harasim, 2004). There are no reports in the literature on the comparison of organically managed swards under continuous grazing use versus those grown for hay; this prompted an interest in the problem and the launching of this study. Based on the results described above, a replicated trial was run aimed at evaluating the yields and the general performance of legume-grass swards of different species composition under pasture vs. hay management.

## Materials and methods

The experiments were carried out in 2009–2012 at the Agricultural Experimental Station operated by IUNG-PIB Grabów (Poland, Masovian voivodeship, 51°21' N, 21°40' E), on a podzolic soil (pgm.gl). A two-factor experiment, arranged as a split-block design with four replications, was laid out on plots each of 60 m<sup>2</sup> in area. The studied factors were: Factor I – four legume-grass mixtures each containing about 50% of legumes and 50% of grasses: 1 - Trifolium repens (25%) + Trifolium pratense (25%) + Lolium perenne (15%) + Dactylis glomerata (15%) + Festuca pratensis (10%) + Festuca rubra (10%); 2 - T. pratense (50%) + L. perenne (20%) + F. pratensis (20%) + Phleum pratense (10%); 3 - Medicago x varia (50%) + D. glomerata (20%) + F. pratensis (20%) + Ph. pratense (10%); 4 - T. repens (25%) + Medicago x varia (25%) + L. perenne (15%) + D. glomerata (15%) + F. pratensis (10%) + D. glomerata (15%) + F. pratensis (10%) + D. glomerata (15%) + F. pratensis (20%) + D. glomerata (15%) + F. pratensis (20%) = 0.5\%

area of 0.5 ha, with no cover crop. In the summer of the year of sowing, the mixtures were mown to eliminate weeds. Then, in the first decade of October, 56 cows were grazed in the pasture area of the experiment, and the herbage was harvested on the treatments set aside for the alternating hay/pasture management. In the autumn, the mixtures were fertilized with P (phosphate powder) at 93 kg ha<sup>-1</sup> and K (potassium sulphate) at 70 kg ha<sup>-1</sup>. In the second year, in autumn (2011), the mixtures were treated with composted manure at 18 t ha<sup>-1</sup>.

During the growing season in the first and second year of production, 56 cows were put to 56 grazing sessions, and the area was mown (in accordance with the layout of the experiment). Before harvest, a 7.2 m<sup>2</sup> area of each hay plot was measured for the yields of green and dry matter. The dry weight of herbage ingested by the cows was calculated as the difference in dry matter (DM) yield from each plot minus the weight of uneaten grass left by the cows. The statistical analysis was performed using DM yield and DM intake by cows, using Tukey's test at a significance level  $\alpha = 0.05$ .

#### **Results and discussion**

In the first year of production, under grazing management a significantly higher DM yield was obtained from the mixture of  $Mv(50\%) + Dg(20\%) + Fp(20\%) + Php(10\% \text{(mixture 3) in comparison with the other mixtures (Table 1). In the subsequent year, the sward of that mixture (mixture 3) and <math>Tr(25\%) + Mv(25\%) + Lp(15\%) + Dg(15\%) + Fp(10\%) + Fr(10\%) \text{(mixture 4)}$  gave the highest output of herbage to be grazed by cows. Variation of yield depending on the species composition of a mixture is known from other studies (Gaweł, 2000). In both years of production, the least efficient mixture was that of Tp(50%) + Lp(20%) + Fp(20%) + Php(10%) (mixture 2) (Table 1). A lower productivity of red clover-grass sward, compared to alfalfa-with-grasses, is in line with the results of other studies (Gaweł, 2009).

	Dry matt	$ar(t ha^{-1})$	Dry matter consumer by			
Laguna grage mintures	Diy man	ci (t ila	cows			
Legume-grass mixtures	1st year of	2 <sup>nd</sup> year of	1st year of	2 <sup>nd</sup> year of		
	utilization	utilization	utilization	utilization		
$1 - Tr^* (25\%) + Tp (25\%) + Lp (15\%) + Dg$ (15%) + $Fp (10\%) + Fr (10\%)$	7.83ab**	8.06ab	6.01a	7.07ab		
2 - Tp (50%) + Lp (20%) + Fp (20%) + Php (10%)	7.28a	7.11a	5.83a	6.34a		
3 - Mv (50%) + Dg (20%) + Fp (20%) + Php (10%)	8.90b	9.36b	6.10a	8.04b		
4 - Tr (25%) + Mv (25%) + Lp (15%) + Dg (15%) + Fp (10%) + Fr (10%)	7.96ab	9.27b	6.20a	8.31b		

Table. 1. Dry matter yields from a ley under grazing management and herbage weight consumed by cows depending on botanical composition of the sward (t ha<sup>-1</sup>)

\* Tr - Trifolium repens; Tp - Trifolium pratense, Lp - Lolium perenne; Dg - Dactylis glomerata; Fp - Festuca pratensis; Fr - Festuca rubra; Php - Phleum pratense; Mv - Medicago varia. \*\* values followed by the same letters do not differ significantly.

In the first year the cows consumed about 6 t ha<sup>-1</sup> of dry weight, regardless of the species used in the legume-grass mixtures (Table 1). In the following year the lucerne-grass mixture (mixture 3), and white clover-grass mixture (mixture 4) were preferred to red clover-grass mixture (mixture 2). High intake of the herbage from stands of alfalfa-with-grass those of white clover-with-grasses was reported in earlier publications (Gaweł, 2000; Harasim, 2004).

	Dry m	atter	Dry matter consumer by cows				
Using	(t ha	<b>1</b> <sup>−1</sup> )	$(t ha^{-1})$				
	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year			
Pasture	8.24a**	8.85a	5.06a	7.02a			
Hay/pasture	7.75a	8.04b	7.01b	7.86b			

Table. 2. Dry matter yields from a ley under grazing management and herbage weight consumed by cows depending on botanical composition of the sward averaged across production years (t ha<sup>-1</sup>)

\*\* values followed by the same letters do not differ significantly.

Initially, the yields of the mixtures under both grazing and the alternating pasture/hay management were similar and stayed at a level of 8 t ha<sup>-1</sup> (Table 2). In the second year, the pasture sward yielded significantly better than the one that alternated hay production with grazing. These results are consistent with those previously obtained by Kitczak *et al.* (2000), where a pasture gave up to 16% more DM yield than a meadow under an alternating pasture/hay management. Harasim (2004) obtained different results, since a continuous grazing produced a 14% decline in forage yield compared with the results from the treatments with hay/pasture. When pasture was alternated with hay, intake of herbage dry matter by the cows was 7.01 t ha<sup>-1</sup> and this was significantly higher than that under the grazing system (Table 2). Similarly, the trend continued in the second year when cows ingested 7.86a t ha<sup>-1</sup> of the herbage from hay/pasture treatments, compared with 7.02b t ha<sup>-1</sup> from the grazed swards.

#### Conclusions

Under the hay/pasture management of a legume-grass ley, there was a drop of about 9% in annual dry matter yield, and the yield of herbage ingested by the cows increased by about 38% as compared to those under the grazing management. The mixtures of Mv (50%) + Dg (20%) + Fp (20%) + Php (10%) (mixture 3) and Tr (25%) + Mv (25%) + Lp (15%) + Dg (15%) + Fp (10%) + Fr (10%) (mixture 4) were characterized by the highest and the most consistent yields. Under the conditions of the experiment it was shown that that short term grass leys on an organic dairy farm should be sown with lucerne-grass mixtures: Mv (50%) + Dg (20%) + Fp (20%) + Php (10%) (mixture 3) and with white clover-grass mixtures: (Tr (25%) + Mv (25%) + Lp (15%) + Dg (15%) + Fp (10%) (mixture 4). Such leys should be managed as pastures.

#### References

Gaweł E. (2000) Evaluation of the suitability of alfalfa-grass mixtures for grazing. P I. Yields and botanical composition. *Pamiętnik Puławski* 121, 66–82.

Gaweł E. (2009) Yielding of red clover and lucerne mixtures with grasses on the ecological farms. *Journal of Research and Applications in Agricultural Engineering* 54(3), 79–85.

Harasim J. (2004) Effect of the change in sward management on the yields of pasture mixtures on arable lands. *Pamiętnik Puławski* 137, 47–58.

Kitczak T., Czyź H. and Gos A. (2000) Floristic composition, yield and chemical composition of pasture and meadow sward of alternating use. *Zeszyty Naukowe Akademii Rolniczej Kraków* 368, 73, 137–143.

# Effect of the frequency of cutting of wolf plants on the yield of a legume-grass sward on an organic farm

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## Abstract

The objective of the trial was to assess the impact of the frequency with which wolf (larged ungrazed) plants are cut on the productivity and botanical composition of legume grass swards under organic management. The study included four periods of cutting wolf plants: 1 - wolf plants left uncut; 2 - wolf plants cut after the first grazing event, 3 - wolf plants cut after the last grazing event; 4 - wolf plants cut after each grazing event. Two-year observations showed a negative impact of frequent cutting of wolf plants after each grazing on pasture productivity. There were no significant differences of dry matter consumed by cows depending on the timing and intensity of cutting wolf plants, but there was a better use of pasture sward in a combination with cutting wolf plants after each grazing.

Keywords: organic farming, legume-grass mixture, pasture utilization, frequency of cutting wolf plants, pasture utilization

## Introduction

The term 'wolf plants' refers to plants that are left ungrazed or partly grazed after a period of grazing (Romo *et al.*, 1997). In previous studies on pastures with lucerne, sainfoin, and white clover in mixture with grasses, wolf plants were cut after each grazing (Harasim, 2004; Gaweł, 2006). In this study, a lot of unconsumed plants in the form of woody shoots, especially lower parts of shoots of legumes, were left by cows after their first grazing, a period that is characterized by a high efficiency of green fodder meant for grazing. So the question is: how to deal with wolf plants left in the pasture field.

The objective of the trial was to assess the impact of the frequency and period of cutting of wolf plants on the productivity and botanical composition of grass-legume swards under organic management.

## Materials and methods

The study was performed at the Agricultural Experiment Farm at Grabów, Poland, province of Mazowieckie, 51°21' N; 21°40' E, on a luvisol that was rated, in terms of agricultural suitability, as a very good rye complex (4). The study period was 2009 to 2012. A monofactorial experiment was laid out as a mirror image design with 4 replicates on 60 m<sup>2</sup> plots. We compared 4 dates of cutting of 'wolf' plants in a sward of *Trifolium pratense* (25%) + *Medicago x varia* (25%) + *Lolium perenne* (20%) + *Dactylis glomerata* (10%) + *Festuca pratensis* (10%) + *Phleum pratense* (10%) after being grazed by cows. The treatments included four dates of cutting wolf plants: 1 – left uncut; 2 – cut after the first grazing event, 3 – cut after the last grazing event; 4 – cut after each grazing event.

The pasture mixtures were sown in the 3<sup>rd</sup> decade of April 2009, without a cover crop. They were subsequently cut in June and in July to eliminate weeds. In the autumn of 2009, 56 cows grazed the total area of the trial. Phosphorus and potassium fertilization was applied at rates of 93 kg P ha<sup>-1</sup> and 70 kg K ha<sup>-1</sup>, respectively. In the autumn of the second year (2010) 18

t ha<sup>-1</sup> of composted manure was applied on the grassland. The mixed sward was grazed four times by a commercial herd of 70 cows (rotational grazing with a progressive limited access to successive parts of a paddock). Before grazing, the weight of fresh herbage was determined from an area of 7.2 m<sup>2</sup> on each plot, and two samples were collected from each plot to measure the dry matter content and to determine the proportion by weight of legumes, grasses, weeds and old stubble in the sward.

#### **Results and discussion**

The time of cutting of wolf plants had a significant effect on dry matter (DM) yield (Table 1). In the first year, DM yield was significantly higher in treatments 1 (uncut), 2 and 3 (cut after first or last grazing period) compared to treatment 4 (cut after each grazing; about 35% decrease). Similarly, in the following year, the lowest DM yield was obtained in frequently cut plots (Table 1). According to Caputo (1977) both the frequency, and also the height at which wolf plants are cut, are important, and when wolf plants were cut at 6–9 cm, green leaves left by animals were cut as well, reducing photosynthesis. This weakens the sward, delays and impedes plant regrowth and ultimately leads to lower yield levels. Other investigators demonstrated that cutting and removal of wolf plants from a permanent pasture and their shredding at certain time intervals is beneficial as it increases utilization under grazing (Romo *et al.*, 1997).

In the present study, the cows consumed less than half of the available biomass, and a similar amount of dry matter, irrespective of the date of cutting of wolf plants (Table 1). Thus the swards were grazed by animals to different degrees, with the lowest average annual utilization rate found in a treatment with uncut wolf plants in 2010 and 2011, where animals had reluctantly consumed a sward with a large amount of old, unconsumed plants from the previous period (Table 1). Such a low usage of legume-grass mixtures in the first grazing, despite the large available forage biomass for grazing cows, was previously observed by Gaweł (2006). In our trial, cutting wolf plants once per year, after the first or the last grazing event, increased the utilization of the pasture. The frequent cutting of wolf plants after every grazing led to a better use of the grass-legume sward (Table 1).

Dates of cutting wolf plants	DM(t	t ha-1)	DM cons	sumed by ws	DM consumed by cows in total dry matter yield (%)					
	year of utilization									
	1 <sup>st</sup>	$2^{nd}$	1 <sup>st</sup>	$2^{nd}$	1 <sup>st</sup>	2 <sup>nd</sup>				
1. Left uncut	16.12a‡	13.29a	7.39a	9,12a	45.8	68.7				
2. Cut after the 1 <sup>st</sup> grazing event	13.94a	13.60a	6.52a	9.60a	47.7	70.6				
3. Cut after the last grazing event	15.99a	12.21ab	8.09a	9.01a	50.6	73.8				
4. Cut after each grazing event	9.92b	10.92b	5.65a	8.07a	56.9	73.9				

Table 1. Yield of the dry matter and dry matter consumed by cow as affected by the frequency of cutting wolf plants and mean year of the degree dry matter consumed by cow (%) of years of utilization

‡ – values within a column followed by the same letter do not differ significantly.

Botanical composition was also affected by the management of wolf plants (Figure 1) with larger weed infestation in uncut plots and a considerable amount of unconsumed and aged plant residues, mainly legume shoots left by cows. In such a sward, as a result of shading, the development of weeds was limited during the following year but the weight of the unconsumed sward increased, and the same was true of the treatment involving a single

cut of wolf plants. The most balanced contribution of legumes and grasses, and low weed infestation, was obtained when wolf plants were cut after each grazing.



 $\square$  – legume plants;  $\square$  – grasses;  $\blacksquare$  – weeds;  $\blacksquare$  – wolf plants;  $0^{**}$  – year of sowing, I-IV grazing Figure 1. Percent contribution of major floristic groups to herbage yields of the pasture mixture according to the frequency of cutting wolf plants and age of sward in two years of utilization

#### Conclusions

In the two years of production, the highest yields (dry biomass) were obtained from plots where wolf plants were left uncut or cut once during the season, with a 30% decrease of yield when wolf plants were cut after the fourth grazing period. Differences were more important in the first year. Cutting wolf plants on a pasture area only once, regardless of the time (after the first or the last grazing) enhanced the use of the pasture sward. The best use of the sward was obtained when wolf plants were cut after every grazing. Leaving the wolf plants uncut or cutting them only after the last grazing led to unfavourable changes in the mixture sward, with increased weed infestation and an accumulation of ungrazed old plants in the second year. Moreover, a high proportion of weed and woody residues in the sward discouraged cows from consuming the herbage. Cutting of wolf plants after each grazing event causes a significant decrease in the productivity of the sward in comparison with the results achieved for other timing regimes adopted in managing wolf plants in the sward.

#### References

Caputa J. (1977) Faucher les refus de pâturage oui! mais. Fourrages-ACTUALITE 17, 25-26.

Gaweł E. (2006) Effect of long-and short-time grazing on the yields and pasture utilization of lucerne cv. Maxi Graze grown in mixture with cocksfoot and sainfoin. *Fragmenta Agronomica* 3(91), 209–221. Harasim J. (2004) Effect of change in sward management on the field of pasture mixtures on arable lands. *Pamiętnik Puławski* 137, 46–58.

Romo J.T., Tremblay M.E. and Barber D. (1997) Are there economic benefits of accessing forage in wolf plants of crested wheatgrass? *Canadian Journal of Plant Science* 77, 367–371.

# Yield performance of binary mixtures of perennial ryegrass cultivars under simulated grazing

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## Abstract

The objective of the experiment was to evaluate the dry matter (DM) performance of monocultures and binary mixtures of perennial ryegrass cultivars under a simulated grazing management in the first harvest year. Four perennial ryegrass cultivars were grown as monocultures and binary mixtures under a simulated grazing management. The four cultivars sown were, AberMagic (AM; diploid), Greengold (GG; tetraploid), Dunluce (DL; tetraploid), and Astonenergy (AE; tetraploid). Binary mixtures comprised of each tetraploid being sown at 15%, 30%, 50%, 70% and 85% of a mixture with AM. Dry matter yield (DMY) was measured eight times from March to November. There was an effect of treatment (cultivar sowing proportion) on DMY (P < 0.05) in spring, summer and autumn and on total DMY. Total DMY of the monocultures were: 13.24 t DM ha<sup>-1</sup> (AM) 12.86 t DM ha<sup>-1</sup> (DL), 11.73 t DM ha<sup>-1</sup> (GG) and 11.36 t DM ha<sup>-1</sup> (AE). The DMY of binary mixtures was related to the inclusion rate in the sown mixture of their component cultivars.

Keywords: perennial ryegrass, cultivar, yield, mixture

## Introduction

Tetraploid perennial ryegrass (*Lolium perenne*) cultivars are generally more palatable and of higher quality (Wilkins, 1991) than diploid cultivars, but with lower tiller densities. The use of tetraploid cultivars has steadily increased from less than 20% in the early 1980's to over 30% today (Grogan and Gilliland, 2010). National and recommended list trials evaluate the performance of cultivars as monocultures, however, it is standard industry practice to sow cultivars as mixtures. There has been a reported advantage of mixtures in terms of enhanced dry matter yield (DMY) over their constituents grown as monocultures (Nyfeler *et al.*, 2009) in species rich swards. However Culleton *et al.* (1986) reported no advantage of mixtures of diploid perennial ryegrass over their components grown as monocultures. There is little information on the effect of mixing cultivars of different ploidy. The objective of the experiment was to evaluate the seasonal and total DMY of monocultures and binary mixtures of perennial ryegrass cultivars under a simulated grazing management in the first harvest year.

## Materials and methods

Fifty seven plots  $(1.5 \text{ m} \times 5 \text{ m})$  were sown in August 2010 at Moorepark Research Centre Fermoy, Co Cork, Ireland  $(50^{\circ}09^{\circ}\text{N}; 8^{\circ}16^{\circ}\text{W})$  in a randomised block design (three replicates). Four perennial ryegrass cultivars, one diploid cultivar AberMagic (AM) (heading date (HD) 27 May) and three tetraploid cultivars Greengold (GG) (HD 29 May), Astonenergy (AE) (HD 31 May) and Dunluce (DL) (HD 28 May), were sown in monoculture and in binary mixtures. The binary mixtures consisted of each tetraploid cultivar sown with AM at the following proportions of tetraploid to diploid: (85:15, 70:30, 50:50, 30:70 and 15:85). A simulated grazing management (8 harvests) was imposed; plots were harvested at 3–4 weekly intervals from March to October. Dry matter yield (>4 cm) was calculated from each plot at each cutting date with a motorised reciprocating blade (Etesia). All mown herbage from each plot was collected and weighed. A sub-sample of 0.1 kg fresh weight of the herbage from each strip was dried for 48 hours at 40°C for DM determination. Following each harvest 35 kg of N/ha was applied with the exception of the final harvest. Statistical analysis was carried out using PROC Mixed in SAS (2003) block and treatment (cultivar sowing proportion) was included in the model.

#### Results

Seasonal and total DMY of the monocultures and mixtures are presented in Table 1. AberMagic and DL had the highest total DMY of the four monocultures. The total DMY of the mixtures was within the range of their components production as monocultures. Treatment had a significant effect (P < 0.001) on total DMY. For example the AM85/GG15 had a higher yield in comparison to AM70/GG30 while AM30/DL70 outyielded AM70/DL30. In the AM/AE binary mixtures reducing the AM proportion reduced the DMY, AM85/AE15 outyielded AM15/AE85 by 1.15 t DM ha<sup>-1</sup>.

	Spring	Summer	Autumn	Total
AM	1.73	9.02	2.48	13.24
GG	1.17	8.40	2.16	11.73
DL	1.25	9.27	2.34	12.86
AE	1.04	8.20	2.12	11.36
AM85/ GG15	1.29	8.75	2.34	12.37
AM70/ GG30	1.29	8.22	2.36	11.87
AM50/ GG50	1.34	8.51	2.38	12.23
AM30/ GG70	1.28	8.44	2.01	11.73
AM15/ GG85	1.25	8.84	2.25	12.33
AM85/ DL15	1.39	8.67	2.38	12.44
AM70/ DL30	1.35	8.67	2.32	12.34
AM50/ DL50	1.48	9.05	2.38	12.91
AM30/ DL70	1.46	9.39	2.42	13.27
AM15/ DL85	1.09	9.06	2.42	12.59
AM85/ AE15	1.46	9.08	2.29	12.82
AM70/ AE30	1.25	8.52	2.31	12.07
AM50/ AE50	1.42	8.84	2.30	12.56
AM30/ AE70	1.15	8.53	2.15	11.83
AM15/ AE85	1.08	8.44	2.15	11.67
Treatment	P < 0.001	P < 0.01	P < 0.05	<i>P</i> < 0.001
SED	13.3	51.8	19.0	66.7

Table 1.	Seasonal	and total	yields	(t DM	ha-1)	of 1	monoculture	s and	binary	mixtures	of	perennial
ryegrass	in the firs	t harvest	year									

AM – AberMagic; GG – Greengold; DL – Dunluce; AE – Astonenergy.

SED - Standard error of difference; Treatment - sown proportion of the cultivars.

Treatment influenced seasonal DMY (P < 0.05), the DMY tended to reduce when the highest yielding monoculture made up a minority of the seed mixture. In the spring period AM was the highest yielding monoculture. The AM85/DL15 mixture yielded more than AM15/DL85. Similarly, AM85/AE15 outyielded AM15/AE85. In the summer period AM and DL were the highest yielding monocultures. The AM30/DL70 had a higher DMY in comparison to AM85/

DL15 mixture. In the AM/AE mixtures a reduced proportion of AM lead to a reduced DMY, AM85/AE15 outyielded AM15/AE85 In the autumn period AM produced a higher DMY than GG and AE. The AM 30/GG70 mixture produced the lowest DMY of the five AM/GG mixtures. Under yielding occurred only once the AM15/DL85 mixture in the spring period produced significantly less than the lowest yielding component.

#### Discussion

The results of the current study are in agreement with Culleton et al. (1986) that there was no benefit of mixtures of perennial ryegrass over their components grown as monocultures. Nyfeler et al. (2009) noted that sufficient diversity between components in mixtures is required for synergism to occur. The absence of synergism between the cultivars may be due to insufficient diversity between the cultivars in the current study despite cultivars of different ploidy in the mixtures. Including cultivars of diverse maturity may add greater diversity to perennial ryegrass seeds mixtures. However, this may lead to increased difficulties in grazing management due to a longer sward reproductive period, which is undesirable for maintaining a high quality sward during the main grazing season. The reproductive period of cultivars leads to increased stem material in the sward which has negative effects on animal production (Gowen et al., 2003). As industry practice is to minimise the range in heading date of seed mixtures the current study is an accurate representation of the sward dynamics that occur in commercial binary seed mixtures. The total DMY of all the mixtures was never less than that of the lowest yielding component. Only one mixture in the spring period produced a yield significantly lower than that of its lowest yielding component, suggesting under yielding is unlikely to occur in binary mixtures. Mixtures may offer greater yield stability in comparison to monocultures due to greater environmental stability as reported by Culleton et al. (1986). The DMY performance of binary mixtures depended on the cultivars used and the inclusion rate of the cultivars in the mixture.

#### Conclusions

No synergism was observed between perennial ryegrass cultivars of different ploidy grown in binary mixtures. The performance of these binary mixtures was largely predicted from the performance of their components grown as monocultures adjusted for the sown proportion of the component cultivars. Seed mixtures should be constructed using cultivars from national recommended lists, as their agronomic performance has been proven in field trials.

#### References

Culleton N., Murphy W.E. and O'Keffe W.F. (1986) The role of mixtures and seeding rate in ryegrass productivity. *Irish Journal of Agricultural Research*, 25, 299–306.

Gowen N., O'Donovan M., Casey I., Rath M., Delaby L. and Stakelum G. (2003) The effect of grass cultivars differing in heading date and ploidy on the performance and dry matter intake of spring calving dairy cows at pasture. *Animal Research*, 52, 321–336.

Grogan D. and Gilliland T.J. (2010) A review of perennial ryegrass variety evaluation in Ireland. O Donovan, M and Hennessy, D (eds) Grasses for the future. Teagasc, Cork, Ireland, pp. 99–116.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Luscher A. (2009) Strong mixtures effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology*, 46, 683–691.

SAS, (2003). SAS Institute. Cary, NC, USA

Wilkins P.W. (1991) Breeding perennial ryegrass for agriculture. Euphytica, 52, 201-214.

## Yield stability of *Festulolium* and perennial ryegrass in southern and central Sweden

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## Abstract

This study investigated yield stability over three years of *Festulolium* species and perennial ryegrass (*Lolium perenne* L.) under the agro-ecological conditions of southern and central Sweden using 26 trials from the official variety testing of forage crops in these regions in the period 2006–2010. The first harvest in each year was used as a measurement of yield stability. In southern Sweden, the decline in dry matter yield at first harvest (DMYFH) over the three years was largest in perennial ryegrass, but significantly less in loloid *Festulolium*. Yield of festucoid *Festulolium* did not decrease and had a significantly different (P < 0.001) slope coefficient to the other species. In central Sweden, there were no significant differences in DMYFH between varieties and species in the third year, but festucoid *Festulolium* suffered significantly less yield decline than the other two species. The *Festulolium* varieties behaved differently between the two regions, mainly because of greater winter damage with increasing latitude. Perennial ryegrass displayed similar behaviour in both regions.

Keywords: *Festulolium, Lolium perenne*, yield stability, sustainability, productivity, dry matter yield

## Introduction

Temporary grass leys in Sweden are mainly established in the spring and cut two-three times over a period of two-four years (Søegaard *et al.*, 2007). Due to intensification of forage production, species with high nutrient quality, such as loloid *Festulolium* and perennial ryegrass, are used in preference to species such as meadow fescue (*Festuca pratensis* L.) and timothy (*Phleum pratense* L.). Because the cultivation area in Sweden extends up to 60° latitude and loloid *Festulolium* and perennial ryegrass are not fully adapted to the Swedish climate, their capacity for winter survival limits their use. A successive decline in biomass production over years has been reported for these species (Gutmane and Adamovich, 2008). Two types of *Festulolium* were examined in this study: the loloid type (*Lolium multiflorum* crossed with *Festuca arundinacea*). The objective of the study, which used data from official variety testing trials, was to investigate productivity and sustainability over three years in different varieties of *Festulolium* and perennial ryegrass under the agro-ecological conditions of southern and central Sweden. These species were chosen because they are vulnerable to winter damage in the study areas. The starting hypothesis was that prolonged testing would reveal differences in yield stability between varieties and species.

## Materials and methods

The Swedish VCU (Value of Cultivation and Use) testing programme was extended to include a third harvesting year on the sites shown in Table 1. The 26 three-year trials included were of a single-factor, randomised block design with three replicates, and were harvested three times in the first and second harvest years. In the third harvest year, in 2006–2008, two cuts were taken and in 2009–2010 three cuts were taken. The 10 varieties examined in the study were selected because they had the highest occurrence in the third year of the ley among all

the varieties in the trials (on average, they occurred in 10 trials in the southern region and in three trials in the central region). The varieties used in the trials changed every year and so the control variety was changed in 2007 from Helmer to SW Birger. Plots were fertilised with 100, 80 and 60 kg ha<sup>-1</sup> N before cuts 1, 2 and 3, respectively, and P and K were applied according to plant-available levels in the soil. The last winter (2009-2010) had on average lower temperatures and longer duration of snow cover than the four previous winters at all sites. The trials were established in spring, and undersown with a cover crop. Seed rate was 800 germinable seeds m<sup>2</sup> for tetraploid perennial ryegrass and loloid Festulolium, 900 seeds m<sup>2</sup> for festucoid Festulolium and 1100 seeds m<sup>2</sup> for diploid perennial ryegrass. All varieties were harvested at the same time, with a stubble height of about 5 cm. The first cut was taken when the perennial ryegrass control variety SW Birger was heading, i.e. when half the panicle was visible on 50% of shoots. Averaged over all 26 trials, the first cut was taken on 9 June, with a range from 25 May to 19 June. Of the varieties shown in Table 2, Gunne, Helmer and SW Birger were bred in Sweden and the others were bred in more southerly locations. The average plot size was 12 m<sup>2</sup> and all plots were harvested using a Haldrup plot harvester with 1.5 m working width. The herbage removed was weighed, sub-sampled and dried for 3 h at 105°C for dry matter (DM) determination.

Site	Coordinatos	Dagion		Total auto				
Sile	ite Coordinates Region -		2006	2007	2008	2009	2010	Total cuts
Tommarp (LB)	55°32′N, 14°15′E	Southern Sweden	1	1	1			3
Svalöv (MS)	55°54′N, 13°08′E	Southern Sweden			1			1
Tvååker (NN)	57°02′N, 12°23′E	Southern Sweden	1	1	1	1		4
Tenhult (F)	57°14′N, 14°17′E	Southern Sweden	1	1	1		1	4
Rådde (PS)	57°36′N, 13°16′E	Southern Sweden	1	1	1	1	1	5
Bjertorp (RS)	58°16′N, 13°06′E	Southern Sweden	1		1			2
Skänninge (ES)	58°27′N, 15°16′E	Southern Sweden		1				1
Lillerud (SS)	59°24′N, 13°13′E	Central Sweden	1					1
Uppsala (CX)	59°50′N, 17°42′E	Central Sweden	1	1	1	1	1	5
Total			7	6	7	3	3	26

Table 1. Study sites in southern and central Sweden with three years of ley, 2006-2010

The letters in brackets after the site is the official abbreviation for that site

Each harvest was statistically analysed using variety as a fixed factor, and calendar year and site within year as random factors. Least squares means were calculated over site and calendar year. Slope coefficients were calculated from a linear regression based on yields over three years for each variety in southern and central Sweden. The Mixed procedure and the Reg procedure were used in the statistics programme SAS Version 9.3 (Littell *et al.*, 2006).

#### **Results and discussion**

Yield at first harvest was chosen to evaluate yield stability, since it gives a good expression of the additional effect of winter damage. To some extent, growth rhythm can also have an influence, e.g. in the late varieties Herbie and Herbal. In the first- and second-year leys, regrowth was about the same (data not shown) as the first growth shown in Table 2.

In southern Sweden, the decline in dry matter yield at first harvest (DMYFH) over three years, described by the slope coefficient, was largest in perennial ryegrass, but significantly less (P < 0.001) in loloid *Festulolium* (Table 2). Yield of festucoid *Festulolium* did not decrease and had a significantly different (P < 0.001) slope coefficient than the other species. In the third year, the festucoid variety Hykor had the significantly largest DMYFH, indicating the high yield stability of this species. The loloid *Festulolium* varieties Paulita

and Perun had significantly larger DMFYH in the third year than all the perennial ryegrass varieties, but the two species had the same decline in yield. There were no differences in yield between the varieties of perennial ryegrass in the third year. In central Sweden, there were no significant differences in DMYFH between varieties and species in the third year, but the festucoid *Festulolium* had significantly less decline (P < 0.001) than the other two species (Table 2). The loloid and festucoid *Festulolium* varieties behaved differently between the two regions, mainly because of greater winter damage in central Sweden. Perennial ryegrass displayed the same behaviour in the two regions. The linear regression of the yield decline in festucoid *Festulolium* had a lower R<sup>2</sup> value in both regions, indicating larger variation in that species.

		Area and ley year										
Variates	Tuna	Sout	hern Sw	veden		Central Sweden						
variety	Туре	1	2	3	Slope coeff.	$\mathbb{R}^2$	1	2	3	Slope coeff.	$\mathbb{R}^2$	
Aberdart (2n)	Lp	7 094	4 941	4 373	-1 361	0.90	6 010	4 080	2 953	-1 529	0.98	
Gunne (2n)	Lp	7 222	5 289	4 123	-1550	0.98	6 732	5 850	3 367	-1 683	0.93	
Helmer (4n)	Lp	7 159	5 069	4 260	-1 450	0.94	6 351	5 614	3 373	-1 489	0.92	
Herbal (4n)	Lp	6 660	4 948	3 725	-1 468	0.99	6 102	4 842	2 912	-1 595	0.99	
Herbie (2n)	Lp	6 768	4 583	3 942	-1 413	0.91	5 904	4 597	2 563	-1 671	0.98	
Hykor, festulolium	Lm x Fa	6 180	6 925	6 824	322	0.63	5 122	6 610	3 142	-990	0.32	
Loporello (4n)	Lp	6 633	5 150	4 135	-1 249	0.99	6 016	5 578	2 997	-1510	0.86	
Paulita, festulolium	Lm x Fp	7 586	5 793	5 759	-914	0.76	6 793	5 142	3 262	-1 766	1.00	
Perun, festulolium	Lm x Fp	7 243	5 479	5 416	-914	0.78	6 856	5 562	4 110	-1 373	1.00	
SW Birger (4n)	Lp	7 303	5 402	4 570	-1 367	0.95	7 263	6 094	3 845	-1709	0.97	
Mean		6 985	5 358	4 713	-1 136	0.88	6 315	5 397	3 253	-1 531	0.89	
LSD		509	426	692			1 293	902	1 570			
CV%		9.1	10.2	13.5			6.4	11.2	24.4			
Р		0.001	0.001	0.001	0.011		0.003	0.001	0.693	0.001		

Table 2. Least square means of dry matter yield (kg ha<sup>-1</sup>) at first harvest during three harvest years in the two regions in Sweden. Linear regression performed on three years

LSD = least significant difference at P < 0.05, 2n = diploid, 4n = tetraploid, Lp = *Lolium perenne* L., Lm = *Lolium multiflorum* Lam., Fa = *Festuca arundinacea* Schreb. and Fp = *Festuca pratensis* Huds.  $R^2 =$  correlation coefficient. The *P*-value for slope coefficient is between species.

#### Conclusions

In general, there was a decline in yield with increasing ley age for varieties of *Festulolium* and perennial ryegrass. Varieties of loloid and festucoid *Festulolium* behaved very differently in southern Sweden compared with central Sweden due to greater winter damage in the latter.

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#### References

Gutmane I. and Adamovich A. (2008) Analysis of *Festulolium* and hybrid ryegrass (*Lolium × boucheanum*) dry matter yield stability. *Grassland Science in Europe* 13, 248–250.

Littell R.C., Milliken G.A., Stroup W.W., Wolfinger R.D. and Schabenberger O. (2006) SAS for Mixed Models. SAS Institute Inc, Cary, NC.

Søegaard K., Gierus M., Hopkins A. and Halling M. (2007) Temporary grassland – challenges in the future. *Grassland Science in Europe* 12, 27–38.

# Plant diversity enhances productivity in grasslands under marginal growing conditions

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#### Abstract

The targeted use of legumes and of functional biodiversity in grassland forage systems is expected to provide major contributions to the challenges of future agriculture. In the current study we analysed the effects of species diversity in agricultural grasslands on productivity and stability in grass-clover mixtures under marginal growing conditions and how these effects are influenced by N fertilisation. Monocultures and 11 different mixtures of two grass species, *Phleum pratense* and *Festuca pratensis*, and two legume species, *Trifolium pratense* and *Trifolium repens*, were established at Korpa Experimental Station, Iceland in spring 2008. The plots received three different N levels per hectare, low (20 kg N), moderate (70 kg N) or high (220 kg N) and were harvested for three years. Increased plant species richness led to greater biomass production and resistance to weed invasion than was expected from the performance of the individual species sown as monocultures. Transgressive overyielding was observed but the diversity effects were reduced at the highest N level. These results have implications for both forage yield and quality and provide interesting opportunities for the production of alternative energy sources from grassland.

Keywords: grass-legume mixtures, transgressive overyielding, net energy production

#### Introduction

To meet the challenges of future agriculture it has been argued that the productivity of current agricultural land has to be improved yet at the same time agricultural resource-efficiency must increase (Foley et al., 2011). Rising prices of fossil fuel and the demand to cut greenhouse gas emissions are forcing the agricultural sector to improve its energy use. As agricultural land is becoming a limited resource it is important to consider net energy production per unit area rather than per kg DM produced (Bertilsson et al., 2008). In grassland-dominated regions, the traditional approach has been to grow monocultures of productive species under high inputs of nitrogen fertiliser. Another way to meet future challenges is to make targeted use of grass-legume mixtures as they have the potential to increase productivity, resource efficiency and forage quality (Peyraud et al., 2009). Recent results of a pan-European network of agro-diversity experiments over 33 sites demonstrate around 30% yield advantage of grass-legume mixtures compared to monocultures of either grasses or legumes (Kirwan et al., 2007). However, these experiments were generally carried out under modest inputs of nitrogen. At one of the sites, though, the yield advantage even persisted at very high nitrogen inputs given that the legume proportion was at least 50% (Nyfeler et al., 2009). The main limitation to the use of legumes in fairly fertile cultivated grassland systems is insufficient persistence of legumes in the sward due to competitive exclusion by their grass companions. Since legumes require relatively high temperatures this problem may be further accentuated under marginal growth conditions in northern areas where winter survival becomes an additional stress factor.

The aim of the current study was to analyse the effects of species diversity on productivity in grass-clover mixtures under marginal growing conditions and how these effects were influenced by nitrogen fertilisation.
### Materials and methods

A total of 66 plots were established in a completely randomised design in spring 2008 at Korpa Experimental Station, Iceland. The set up followed a simplex design (Kirwan *et al.*, 2007) with four monocultures of *Phleum pratense* L., *Festuca pratensis* Huds., *Trifolium pratense* L. and *Trifolium repens* L., and 11 mixtures of the four species differing widely in sowing proportions. Swards were fertilised with 20 kg N (Low), 70 kg N (Moderate) or 220 kg N ha<sup>-1</sup> yr<sup>-1</sup> (High). All plots received 40 kg P and 60 kg K ha<sup>-1</sup> yr<sup>-1</sup> in early spring. The plots were harvested twice a year for three years (2009–2011) to determine dry matter yield and sown and unsown species proportions were determined by manually separating plant samples from permanent sub-plots. Yield data was analysed by multiple linear regression and followed the approach described by Kirwan *et al.* (2009) and Nyfeler *et al.* (2009). The model selected included main effects of all four species, species×fertiliser interactions and grass×legume interaction ( $r^2 = 0.93$ , mean for three years). Estimates of energy parameters were based on information in Bertilsson *et al.* (2008) and McDonald *et al.* (1995).

#### **Results and discussion**

The highest yielding species at Low N was *T. pratense* (4.57 t DM ha<sup>-1</sup> yr<sup>-1</sup>), whereas *F. pratense* was highest yielding at High N (8.72 t DM ha<sup>-1</sup> yr<sup>-1</sup>). The equal stand mixtures yielded on average 72, 52 and 37% more than would be expected from the yield of their components in monoculture at Low, Moderate and High levels of N respectively (Figure 1a). Transgressive overyielding was observed at all N levels and even at the highest N level both the equal stand mixture and the mixture dominated by *F. pratense* outyielded the highest yielding monoculture. The diversity effect increased steadily with time and was 44, 54 and 69% on average over N levels for the equal stand mixture for years 1, 2 and 3 respectively. The consistent overyielding is in line with that observed by Nyfeler *et al.* (2009) and can primarily be explained by the persistency of the legume components in the mixtures at all N levels whereas both legume species survived poorly in monocultures (Figure 1b). The net energy production (total energy produced minus energy input) increased with increased N application, for both grass monocultures (Table 1). This resulted in an energy profit



Figure 1. (a) Predicted total yield (t ha<sup>-1</sup> yr<sup>-1</sup>) and (b) yield of unsown species over three years for monocultures (filled symbols) of *P. pratense* ( $\blacklozenge$ ), *F. pratensis* ( $\blacksquare$ ), *T. pratense* ( $\blacklozenge$ ) and *T. repens* ( $\blacktriangle$ ), mixtures dominated by each of the four species (open symbols) and equal stand mixtures (×) at Low, Moderate and High N levels. Transgressive overyielding is indicated by the dotted line

of 94, 49 and 23% and land savings of 46, 31 and 15% for the mixture at Low, Moderate and High N levels respectively. Thus, a well balanced grass-legume mixture with 70 kg  $ha^{-1}$  N produces about the same net energy as a grass monoculture with 220 kg  $ha^{-1}$  N. It is though worth pointing out that an additional 150 kg  $ha^{-1}$  N increased net energy from the mixture by 24%. Whether environmental costs are too high in that system remains, however, to be seen.

Table 1. Yield and energy parameters for grass monoculture (mean of *P. pratense* and *F. pratensis*) and grass-legume mixture (equal stand) at three levels of N fertiliser

	Lo	w N	Mode	rate N	Hig	h N
	Grass	Mixture	Grass	Mixture	Grass	Mixture
Yield (kg ha <sup>-1</sup> )	3.350	6.160	5.180	7.480	8.040	9.490
Energy use (MJ ha <sup>-1</sup> ) <sup>a</sup>	6.840	6.840	8.940	8.940	15.240	15.240
Gross energy production (MJ ha <sup>-1</sup> ) <sup>b</sup>	61.640	113.344	95.312	137.632	147.936	174.616
Net energy production (MJ ha <sup>-1</sup> )	54.800	106.504	86.372	128.692	132.696	159.376
Energy profit of mixtures (%)		94		49		23
Land savings of mixtures (%)		46		31		15

 $^{a}42 \text{ MJ kg}^{-1} \text{ N}$ ; 6000 MJ ha<sup>-1</sup> for other energy needed (Bertilsson *et al.*, 2008).

<sup>b</sup>18.5 MJ kg<sup>-1</sup> dry matter (total energy) (McDonald *et al.*, 1995).

### Conclusions

A well balanced grass-legume mixture consistently outyielded the highest yielding mixture component in monoculture at all N levels and also resisted the ingression of unsown species. This has implications for both forage yield and quality and provides interesting opportunities for the production of alternative energy sources from grassland.

### References

Bertilsson G., Kirchmann H. and Bergström L. (2008) Energy analysis of organic and conventional agricultural systems. In: Kirchmann H. and Bergström L. (eds) *Organic Crop Production – Ambitions and Limitations*, Springer, 173–188.

Foley et al. (2011) Solutions for a cultivated planet. Nature 478, 337-342.

Kirwan L., Lüscher A., Sebastià M.T., Finn J.A., Collins R.P., Porqueddu C., Helgadóttir Á., Baadshaug O.H., Brophy C., Coran C., Dalmannsdóttir S., Delgado I., Elgersma A., Fothergill M., Frankow-Lindberg B.E., Golinski P., Grieu P., Gustavsson A.M., Höglind M., Huguenin-Elie O., Iliadis C., Jørgensen M., Kadžiulienė Ž., Karyotis T., Lunnan T., Malengier M., Maltoni S., Meyer V., Nyfeler D., Nykanen-Kurki P., Parente J., Smit H.J., Thumm U. and Connolly J. (2007) Evenness drives consistent diversity effects in an intensive grassland system across 28 European sites. *Journal of Ecology* 95, 530–539.

Kirwan L., Connolly J., Finn J.A., Brophy C., Lüscher A., Nyfeler D. and Sebastià M.-T. (2009) Diversity-interaction modeling: estimating contributions of species identities and interactions to ecosystem function. *Ecology* 90, 2032–2038.

McDonald P., Edwards R.A., Greenhalgh J.F.D. and Morgan C.A. (1995) *Animal Nutrition, Fifth Edition.* Longman Scientific & Technical, Harlow, 240 pp.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Lüscher A. (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology* 46, 683–691.

Peyraud J.L., Le Gall A. and Lüscher A. (2009) Potential food production from forage legume-based-systems in Europe: an overview. *Irish Journal of Agricultural and Food Research* 48, 115–135.

# Effect of N fertiliser application rate on herbage production and sward clover content in grazed grass clover plots

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## Abstract

Perennial ryegrass-white clover swards can make an important contribution to sustainable ruminant production systems. White clover use in grazed swards in Ireland has been in decline since the 1970s due to the availability and generally low cost of fertiliser nitrogen (N). In recent times there has been renewed interest in white clover use on farms for environmental and economic reasons. The objective of this experiment is to provide information on the interaction between grass-clover swards and N fertiliser in frequently grazed swards. This experiment was a 2×5 factorial arrangement, with two swards (grass only and grass-clover), and five fertiliser N application rates (0, 60, 120, 180, 240 kg N ha<sup>-1</sup>), with three replicates per treatment. Herbage production and sward clover content were measured in 2010 and 2011. Over the two years the grass-clover swards, on average, produced significantly (P < 0.001) more herbage mass (plus 3236 kg DM ha<sup>-1</sup>) than the grass only swards (9944 kg DM ha<sup>-1</sup>). Treatments receiving 0, 60 and 120 kg N ha<sup>-1</sup> produced significantly (P < 0.05) less herbage than treatments receiving 240 kg N ha<sup>-1</sup>. As N fertiliser application rate increased, the sward clover content declined.

Keywords: fertilization, herbage, grazing, nitrogen, perennial ryegrass, white clover

## Introduction

White clover (*Trifolium repens*) is the most important legume in grazed pastures in temperate regions. It grows well in association with grasses, is tolerant of grazing and its herbage is of high nutritional quality for livestock (Whitehead, 1995). White clover use in grazed swards in Ireland (and across many temperate regions) has been in decline since the 1970s but in recent times there is renewed interest in white clover for environmental and economic reasons. Limits on N-fertiliser use imposed by the EU Nitrates Directive, as well as the increased cost of fertiliser, have focused farmers to look for alternative and lower cost sources of N. A sward with a high white clover content (e.g. 20% of total DM (10 t ha<sup>-1</sup>) per year) can fix about 150 kg N ha<sup>-1</sup> year<sup>-1</sup> (from studies in UK and Ireland). In a frequently defoliated sward it is unclear what rate of N fertiliser N. Therefore, the objective of this experiment was to compare herbage production on grass-clover swards and grass-only swards at a range of N fertiliser application rates, and to examine the effect of N fertiliser application on sward clover content.

## Materials and methods

A 2×5 factorial arrangement experiment replicated three times was undertaken at the Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland. The experiment had two sward types and five annual N fertiliser application rates. A grass-only

sward (50:50 mix of cv.Tyrella and cv. Dunluce sown at 37 kg ha<sup>-1</sup>) and a grass-clover sward (same grass sward plus 2.5 kg ha<sup>-1</sup> each of white clover cultivars Crusader and Chieftain) were sown in May 2009 and rotationally grazed by heifers for the remainder of 2009. Grazing plots (8 m × 8 m) were established in February 2010. Five annual fertiliser rates, 0, 60, 120, 180, 240 kg N ha<sup>-1</sup> year<sup>-1</sup> were randomly assigned across the two swards. Fertiliser application strategy is shown in Table 1. Plots were grazed between February and October; 9 times in 2010 and 10 times in 2011. Pre-grazing herbage mass was measured to a height of 4 cm using an Agria mower. Sward clover content (to 4 cm) was measured on each grazing occasion by separating a sample (c. 100 g) of the pre-grazing herbage mass into clover and grass components, attaining the dry matter (DM) and calculating the proportion of clover in the total herbage mass. Data were averaged across the two years and analysed using the PROC GLM procedure in SAS.

Ferti- liser rate (kg N ha <sup>-1</sup> )	Mid Jan.	Mid Mar.	Mid April	Early May	Late May	Mid June	Early July	Late July	Mid Aug.	Mid Sept.
0	0	0	0	0	0	0	0	0	0	0
60	30	30	0	0	0	0	0	0	0	0
120	28	28	31	16.5	16.5	0	0	0	0	0
180	28	28	31	16.5	16.5	12	12	12	12	12
240	28	28	33	33	33	17	17	17	17	17

Table 1. N fertiliser application strategy

### **Results and discussion**

Average herbage mass was significantly (P < 0.001) greater in year 2 (12542 kg DM ha<sup>-1</sup>) compared to year 1 (10582 kg DM ha<sup>-1</sup>) due to slightly warmer conditions in February (+4.1°C), March (+1°C), April (+2.2°C) and May (+0.5°C) of 2011. Grass-clover swards had significantly (P < 0.001) greater total herbage production (13,180 kg DM ha<sup>-1</sup>) than grass-only swards (9,944 kg DM ha<sup>-1</sup>). Treatments receiving 0, 60, 120, 180 kg N ha<sup>-1</sup> produced significantly (P < 0.05) less herbage than 240 kg N ha<sup>-1</sup> treatments, and the 180 kg N ha<sup>-1</sup> treatments produced significantly (P < 0.05) less herbage than 240 kg N ha<sup>-1</sup> treatments, and the 180 kg N ha<sup>-1</sup> treatments. There was a significant interaction (P < 0.05) between sward type and N fertiliser application rate, due to the greater effect of increasing N fertiliser application rate on grass-only swards compared to the grass-clover swards (Figure 1). Average sward clover content was similar in both years (0.31) across treatments. Sward clover content increased on all treatments during the year and was greatest in the July to October period.



Figure 1. Herbage production on grass only and grass clover swards receiving five rates of N fertiliser application  $ha^{-1}$  year<sup>-1</sup>

Sward clover content was significantly (P < 0.001) lower on the 180 and 240 kg N ha<sup>-1</sup> treatments (average 0.26) compared to the 0 and 60 kg N ha<sup>-1</sup> treatments (average 0.39), and the 120 kg N ha<sup>-1</sup> treatment had significantly (P < 0.05) less sward clover content compared to the 0 kg N ha<sup>-1</sup> treatment.

Grass-clover swards receiving no N fertiliser had similar herbage production to the grassonly swards receiving 240 kg N ha<sup>-1</sup>, indicating that the swards can fix fertiliser at rates similar to, and in some cases greater than, previously reported in the scientific literature. Although the rate of herbage production increase on the grass-clover swards decreased as N fertiliser rate increased, herbage production benefits were observed with N fertiliser application, particularly at the 180 and 240 kg N ha<sup>-1</sup> application rate. Sward clover content was lower on the 180 and 240 kg N ha<sup>-1</sup> treatments than on the other treatments, but was in the range of 35–40% of the herbage mass in the August to October period, similar to the sward clover contents reported by Humphreys *et al.* (2008) in low fertiliser N swards. Frequent tight defoliation of swards is likely to benefit clover production and persistence within grazed swards as clover requires penetration of light to the base of the sward for stolon growth and persistence (Dennis and Woledge, 1987; Caradus and Chapman, 1991).



 $\rightarrow$  0 kg N ha<sup>-1</sup> = 60 kg N ha<sup>-1</sup>  $\rightarrow$  120 kg N ha<sup>-1</sup> = 180 kg N ha<sup>-1</sup> = 240 kg N ha<sup>-1</sup> Figure 2. Average sward clover content between February and October (2010 and 2011) in grass clover swards receiving five rates of N fertiliser application ha<sup>-1</sup> year<sup>-1</sup>

## Conclusions

At high N fertiliser application rates the inclusion of clover in a sward can increase herbage production. Sward clover content was lower at higher N fertiliser rates especially from July onwards. All swards in the experiment, on average over a two-year period, had sward clover contents greater than 0.25. This experiment must be continued for a number of years to quantify clover persistence in fertilised swards.

## References

Caradus J.R. and Chapman D.F. (1991) Variability of stolon characteristics and response to shading in two cultivars of white clover (*Trifolium repens* L.). *New Zealand Journal of Experimental Agriculture* 34, 239–247. Dennis W.D. and Woledge J. (1987) The effect of nitrogenous fertiliser on the photosynthesis and growth of white clover/perennial ryegrass swards. *Annals of Botany* 49, 627–635.

Gilliland T.J., McGilloway D. and Conaghan P. (2009) Characterisation of different white clover (*Trifolium repens* L.) cultivar evaluation systems in Ireland and their relevance to grassland farming. *Irish Journal of Agricultural and Food Research* 48, 227–242.

Humphreys J., O'Connell K. and Casey I.A. (2008) Nitrogen flows and balances in four grassland based systems of dairy production on a clay-loam soil in a moist maritime climate. *Grass and Forage Science* 63, 467–480. Whitehead D.C. (1995) *Grassland Nitrogen*. CAB International, Wallingford, UK.

# Assessment of tracer methods for identifying belowground niche differentiation in grassland

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### Abstract

Benefits of grassland mixtures over monocultures are hypothesised to be based on niche complementarity among plant species. Belowground niche differentiation can be assessed by injecting tracers at different soil depths and comparing the resulting tracer concentrations in plant material of individual species. In previous methods, one single tracer was injected to one soil depth per sub-plot, requiring separate sub plots for each depth of injection (single tracer method). A multiple tracer method in which different tracers are injected each at a distinct soil depth within the same sub-plot has been proposed (multiple tracer method). To be valid, this method has to meet the following requirements: 1) tracers have to be reliable markers of the targeted soil depth, i.e. remain at the injected soil depth throughout the experimental period, and 2) tracers have to allow a quantitative estimate of nutrient uptake. We tested these requirements for four different tracers <sup>15</sup>N, lithium, rubidium and caesium. For the combination of tracers and plant species in this experiment, we were unable to determine a set of two or more tracers that meet both requirements for the multiple tracer method.

Keywords: root activity, tracers, grassland mixtures, niche differentiation

#### Introduction

Greater plant diversity in grassland plant communities can result in increased yields, greater stability in response to disturbance, reduced invasion by weeds and improved nutrient retention. These benefits are suggested to be based on the complementarity between the niches of the different plant species or functional groups.

Belowground niche differentiation among species, i.e. differing root activity at different soil depths, can be assessed by injecting tracers at multiple soil depths and comparing the resulting tracer concentrations in plant material of individual species. Traditionally, this has relied on injecting one tracer at different soil depths, requiring a separate sub-plot for each soil depth (see Figure 1). Fitter *et al.* (1986) proposed a multiple tracer method in which different tracers are each injected at a different soil depth within the same sub-plot. A benefit of this multiple tracer method is that only one sub-plot is required to deliver tracers to several soil depths, and between-sub-plot variability for different soil depths is eliminated. The number of samples for species sorting and tracer analysis in forage is also reduced.

The objective of the current experiment was to investigate if tracers meet the requirements to be used in the multiple tracer method. We selected a number of tracers that have been widely used (<sup>15</sup>N, Cs, Rb, Li), of which Cs, Li and Rb are supposed to represent potassium ions (K<sup>+</sup>). The rate of uptake of different tracers is largely determined by factors such as ion diameter and valency. In addition, there can be strong interactions in uptake between tracers of the same electrical charge. A well known example of cation competition is the uptake of Mg<sup>2+</sup>, which is strongly depressed by K<sup>+</sup> and Ca<sup>2+</sup>. We investigated whether the tracers satisfied the following requirements: 1) to be reliable and persistent markers of the targeted soil depth, and 2) to permit quantitative comparison of root activity of different plant species.

### Materials and methods

The tracers were tested in a field experiment consisting of mixtures of four species: *Lolium perenne* (*Lp*), *Trifolium repens* (*Tr*), *Cichorium intybus* (*Ci*) and Plantago lanceolata (*Pl*). Tracers were injected into a 50 cm  $\times$  50 cm sub-plot in the week following cutting at a rate of 6.3 mmol per plot, to address two objectives. First, to check whether the tracers remained at the depth of injection, different soil depths were injected with Li, Cs, Rb and <sup>15</sup>N. For each depth (5, 15, 25 and 60 cm), 36 holes were predrilled in a grid in such a way that each tracer was delivered to a distinct and known depth. Five weeks after injection, four soil samples of up to 90 cm depth were taken in each of 12 plots. The soil cores were separated into 10 cm horizons, pooled per plot and air dried. Tracer concentrations in plant and soil samples were measured with a mass spectrometer. Second, to test prerequisite 2, a tracer cocktail containing all four tracers was injected at the main rooting depth (10 cm). Aboveground biomass of plants was harvested four weeks after injection and sorted into species. Subsequently, they were dried, ground and analysed for tracers.

### **Results and discussion**

1) Reliable markers of soil depth. Analyses of the distribution of the different tracers throughout the soil profile showed that <sup>15</sup>N, Rb and Cs remained concentrated at their respective injection depths, and thus fulfilled prerequisite 1. Lithium concentrations were not elevated at the injection depth, indicating that it was not stable at the injection site or that background concentrations in the soil were too high or too variable and, therefore, did not fulfil prerequisite 1. Lithium is known to be a leachable element (Mamolos *et al.*, 1995) which seems confirmed by our results, and this would cause problems if used with the multiple tracer technique but also with the single tracer technique.

2) Reliable assessment of root activity. Even though all tracers were injected with the same concentration to the same soil depth, huge differences occurred in both (i) the concentration of different tracers within a species (e.g. [Cs]/[Rb] ratio was 0.1 in *Lp*; Table 1) and (ii) the concentration ratios of these tracer pairs among plant species (e.g. for [Rb]/[Li] from 4.5 in *Pl* to 21.8 in *Lp*; Table 1). Consequently, injection of two such tracers to two different soil depths to quantify root activities at these soil depths would face confounding factors: (i) differing concentrations of the two tracers in a plant species may either be the result of differing root activity among the two soil depths or from differing uptake rates of the two tracers *per se.*, and (ii) differing ratios of tracer concentrations among species may be the result of either a difference in the root activity at the two soil depths between the species or differing tracer uptake ratios of the compared species.

As expected, the ratios of tracer pairs that do not represent the same nutritional element ('Mix', Table 1), showed a wide range between the different species, with up to a 12-fold difference between the lowest (46) and highest (547) ratio for <sup>15</sup>N/Li. The low <sup>15</sup>N concentration in *Tr* compared to for example *Lp* is a direct result of biological N fixation by *Tr*. Therefore, if such a combination was used in a multiple tracer experiment (i.e. N<sup>15</sup> injected at shallow depth, Li injected at deep depth), the conclusion would be that *Tr* would be much more active at deep layers compared to for example *Lp*. Even for the tracers selected because they are similar to K<sup>+</sup>, ('K-analogs', Table 1) there was still up to a two-fold difference among the tracer ratios for different species for the best performing tracer pair (Rb/Cs) (Table 1). Here, the concentration of Rb was approximately 10 times higher than that for Cs. Therefore, using these two tracers at different depths would not allow us to quantify the uptake in a given soil layer, i.e. whether the upper layer provides 20, 50 or 80% of nutrients. However, this would be possible with the single tracer method in separate sub-plots.

Table 1. Tracer concentrations of Cs, Li, Rb (mol kg<sup>-1</sup>) and <sup>15</sup>N (% excess) in plant material of six grassland species, and the ratios between the different tracer pairs. This follows the initial application of equal tracer concentrations at the same depth. Within a tracer pair, bold values indicate the highest ratio and italic values the lowest ratio. Different letters denote significant differences within columns at *P* > 0.05 (ANOVA)

		Tracer concentrations						Tracer ratios <sup>1</sup>						
		K-analogs <sup>2</sup>					K-analogs <sup>2</sup> Miz				Mix			
Species	Cs		Li		Rb	)	<sup>15</sup> N	[	Cs/Rb	Cs/Li	Rb/Li	<sup>15</sup> N/Cs	<sup>15</sup> N/Li	<sup>15</sup> N/Rb
Lp	0.56	а	0.27	а	5.8	а	146	с	0.10	2.1	21.8	260	547	25
Tr	0.71	а	0.49	bc	4.4	а	23	а	0.16	1.5	9.0	32	46	5
Ci	1.04	а	0.44	b	5.2	а	96	b	0.20	2.4	11.8	92	216	18
Pl	0.47	а	0.84	с	3.8	а	145	с	0.13	0.6	4.5	305	173	38

<sup>1</sup>Tracer ratios that are unequal to 1 represent differential uptake of the two tracers by a species. <sup>2</sup>K-analogs: tracers supposed to represent potassium ions (K<sup>+</sup>).



Single tracer method

-Multiple subplots (A, B, C) per experimental unit

-One soil depth per sub-plot

-One tracer ( $\times$ ) for each depth

Multiple tracer method -One sub-plot (A) per experimental unit -Multiple soil depths per sub-plot

-Different tracers  $(\times, 0, \wedge)$  for each depth

Figure 1. Overview of the single (a) and multiple (b) tracer method

### Conclusions

For the combination of tracers and plant species in this experiment, we were unable to determine a set of two or more tracers that meet all requirements for the multiple tracer method. Thus, the multiple tracer technique does not appear to be an alternative to the single tracer method.

### Acknowledgements

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### References

Fitter A.H. (1986) Spatial and temporal patterns of root activity in a species-rich alluvial grassland. Oecologia 69, 594-599.

Mamolos A.P., Elisseou G.K. and Veresoglou D.S. (1995) Depth of root activity of coexisting grassland species in relation to N-addition and P-addition, measured using nonradioactive tracers. Journal of Ecology 83, 643-652.

# Development and growth of grass cultivars in pure stands and in meadow mixtures

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## Abstract

This study aimed to estimate the development, growth speed and yield in the year of sowing of 14 cultivars of 6 grass species – *Dactylis glomerata*, *Festuca pratensis*, *Phleum pratense*, *Lolium perenne*, *Festuca rubra* and *Poa pratensis* – destined for cutting. Until tillering-stage appeared there were large differences between species but small differences between the cultivars. In pure stands, Naki, Bajka and Diament of *L. perenne* and Wanda of *F. pratensis* revealed good development and forage productivity. The development of cvs of *D. glomerata*, *Ph. pratense* and *P. pratensis* in mixtures was evidently slower than in pure stands. The mixtures developed and yielded similarly but much lower compared to their *L. perenne* components in pure stands – Naki and Bajka, especially in the second re-growth.

Keywords: sowing year, grass cultivars, growth, development, yields, pure stands, mixtures

## Introduction

Fast development and tillering are desirable for good establishment and ground cover. Moreover, it is essential to achieve high yields in the year of sowing (Rutkowska *et al.*, 1994; De Vliegher and Carlier, 2002). Unfortunately there is not much information about the development rate of individual grass cultivars in the sowing year (Rutkowska and Janicka, 1992). The aim of the study was the estimation of 14 Polish cultivars of 6 grass species in relation to development, growth rate and yield in the sowing year.

## Materials and methods

The study was carried out in 2010 at the Experimental Station of Agronomy Department of WULS at Ursynów (central Poland). It was situated on mineral brown soil with medium phosphorus, potassium and magnesium concentrations. The soil pH<sub>KCI</sub> was 5.2. The experiment was designed as a randomised complete block with four replications. The size of plots was 1  $m^2$ ; the row spacing was 10 cm. The sowing rates were applied according to the norms recommended in practice. Fourteen cultivars of 6 grass species were tested: Dactylis glomerata (Amera, Berta), Festuca pratensis (Pasja, Wanda), Phleum pratense (Karta, Skala), Lolium perenne (Bajka, Diament, Gagat, Naki), Poa pratensis (Eska-46, Skiz) and Festuca rubra (Reda, Kos). The cultivars were examined in pure stands and in two different mixtures. An early mixture consisted of: Amera (10%), Wanda (30%), Skala (20%), Kos (10%), Naki (20%) and Eska-46 (10%), while the medium early mixture consisted of: Berta (10%), Pasja (30%), Karta (20%), Reda (10%), Bajka (20%) and Skiz (10%). The sowing date was 28 April 2010. The following doses (kg ha-1) of mineral fertilizers were applied: 140 N, 26 P and 100 K. Plant heights were measured every 7-10 days. At 54 days after sowing, plants were taken from a 15 cm long  $\times$  8 cm wide section of row and the development of each of them was noted. One cultivation and two production cuts were made and yields and botanical composition were measured. The ground cover in the rows after each cutting was measured. Data were analysed by one-factor variance analysis and verified using LSD test. The weather conditions during the vegetation period were favourable for growth and development of grasses (Table 1). The dates were recorded at the meteorological station (Ursynów) of Meteorology and Climatology Department of SGGW. That vegetation period according to Vinczeffy (1984) was classified as rainy (0.235).

Month/	Ave	age dail	y air tem	p. (°C)	Sum	monthly	rainfall	(mm)	Ratio of rainfall per °C
decade	Ι	II	III	mean	Ι	II	III	total	$\Sigma mm \cdot \Sigma^{o}C^{-1}$
April	8.3	10.4	10.2	9.6	18.9	7.5	9.4	35.8	0.124
May	12.2	13.7	15.2	13.7	35.9	57.2	48.6	141.7	0.334
June	19.6	17.4	17.3	18.1	85.1	36.4	34.9	156.4	0.288
July	21.1	24.9	20.3	22.0	1.3	25.0	62.4	88.7	0.130
August	21.1	21.2	17.5	19.8	58.9	16.5	54.6	130.0	0.212
September	12.0	13.1	12.0	12.4	111.3	4.7	19.0	135.0	0.363
IV–IX	15	.93±4.6	(mean ±	SD)	687	.6±28.8	(mean ±	SD)	0.235

Table 1. Mean air temperature (°C) and rainfalls (mm) in 2010, recorded at Ursynów meteorological station

### **Results and discussion**

Until the tillering stage appeared there were large differences between species but rather small differences between the cultivars. The earliest species to emerge and achieve tillering stage were L. perenne and F. pratensis (emergence: 9-11, tillering: 26-27 days after sowing) whereas Ph. pratense and P. pratensis were the latest (12–14 and 30–37 days, respectively). There was no emergence of F. rubra Kos. On day-54 after sowing, differences in development phase depended not only on species but also on cultivar. Among L. perenne cvs the fastest was Naki (70.4% of plants formed 4 or more tillers) whilst Bajka was the slowest (11.1%). The seedlings of P. pratensis (especially Eska-46) and Ph. pratense (Karta) developed slowly and 95.3% and 78.6% of them respectively had 3-4 leaves unfolded and only 4.7% and 3.6% started tillering. The earlier cultivars grew faster and were taller on the days of cuts compared with the medium-early cultivars of the same species, but the differences were not significant. Most of them covered soil and yielded better than the later ones (Table 2). They also allowed weeds to develop only to a low extent (Table 3). Naki, Bajka and Diament of L. perenne produced the highest annual vields, which were significantly different to the vields of Ph. pratense Karta and both cvs of P. pratensis. In the mixtures the most competitive were Naki of L. perenne and Pasja of F. pratensis. Those cultivars greatly reduced the establishment of slower species -P. pratensis and F. rubra cultivars. This is in agreement with the other studies (Rutkowska et al., 1994). Those cultivars

Smaailaa	Cultivor	Sward hei	ght (cm)	Cover (%)		Yield (t ha-1)	
species	Cultival	Ι	II	mean	Ι	II	annual
Facture protonois	Wanda	49.4 ef*	65.3 bc	78.8 abc	2.94 bcd	3.38 bcd	6.32 bcd
resiuca praiensis	Pasja	45.8 def	64.1 bc	76.9 ab	2.54 abcd	2.62 abc	5.16 ab
Dactulia alomorata	Amera	60.7 g	65.2 bc	90.6 d	2.86 bcd	2.39 ab	5.26 ab
Daciyus giomeraia	Berta	51.7 fg	67.4 c	78.8 abc	2.33 abcd	2.78 abc	5.11 ab
Dhloum protonco	Skala	40.4 bcde	52.2 a	72.5 a	2.86 bcd	2.43 abc	5.29 ab
r nieum praiense	Karta	29.9 ab	47.5 a	74.4 a	2.49 abcd	2.21 ab	4.69 ab
	Naki	34.4 abc	49.2 a	87.5 cd	3.26 d	4.97 e	8.23 e
I alium manana a	Diament	35.1 abcd	52.2 a	85.6 bcd	2.86 bcd	4.40 de	7.26 cde
Lotium perenne	Gagat	35.7 abcd	48.5 a	82.5 abcd	1.68 a	3.67 cd	5.36 ab
	Bajka	32.6 abc	51.8 a	85.6 bcd	2.66 bcd	5.08 e	7.74 de
Festuca rubra	Reda	37.9 bcd	53.7 a	81.3 abcd	2.25 abc	2.91 abc	5.16 ab
Pog pratansis	Skiz	34.1 abc	55.8 ab	72.5 a	2.23 abc	1.89 a	4.12 a
1 ou praiensis	Eska-46	25.7 a	48.5 a	73.8 a	2.00 ab	2.02 a	4.02 a
Mixture	Early	43.2 cdef	49.0 a	82.5 abcd	3.06 cd	2.94 abc	6.00 bc
Mixture	Medium early	45.4 def	48.9 a	82.5abcd	2.63 abcd	2.63 abc	5.27 ab
LSD <sub>0.05</sub>		10.89	10.23	10.01	0.960	1.276	1.725

Table 2. Sward structure and yields of dry matter of the 13 cultivars in pure stands and two mixtures in I-II cuts

\*values within columns indicated by the same letters are not significantly different.

made the highest share in the yields: *L. perenne* cv. Naki of early mixture (on average 41%) and *F. pratensis* cv. Pasja – in later one (on average 52%). It was also found that their growth rate in mixtures was similar to development in pure stands, whilst the growth rate of *D. glomerata*, *Ph. pratense* and *P. pratensis* cvs in mixtures was evidently slower than in pure stands. The early mixture yielded a little better than the later one. The annual yields of mixtures were considerably lower than their *L. perenne* components – Naki and Bajka cvs. As was stated by De Vliegher and Carlier (2002), rarely do mixtures outyield their most productive component. But contrary results were obtained by Kirwan *et al.* (2007).

		I cut					II cut				
				Sown gras	sses in mix-			Sown gras	sses in mix-		
Species	Cultivar	Sown	Woods	t	ires	Sown	Weeds	tu	ires		
		grasses	weeus	Farly	Medium	grasses	weeus	Farly	Medium		
				Larry	early			Larry	early		
Festuca	Wanda	72.9	27.1	27.2		97.6	2.4	40.0			
pratensis	Pasja	69.4	30.6		40.4	98.4	1.6		63.6		
Dactylis	Amera	79.0	21.0	4.0		96.4	3.6	7.3			
glomerata	Berta	53.9	46.1		2.5	95.1	4.9		4.0		
Phleum	Skala	54.8	45.2	1.8		92.9	7.1	2.7			
pratense	Karta	53.7	46.3		2.3	84.7	16.3		1.1		
	Naki	92.9	7.1	34.1		99.0	1.0	48.5			
Lolium	Diament	89.7	10.3			98.2	1.8				
perenne	Gagat	76.2	23.8			98.3	1.7				
	Bajka	87.7	12.3		27.2	97.5	2.5		26.6		
Festuca	Reda	58.3	41.7		1.7	83.6	16.4		1.9		
rubra	Kos	0.0	0.0	0.0				0.0			
Poa prat-	Skiz	29.9	70.1		0.3	67.7	32.3		0.6		
ensis	Eska-46	41.3	58.7	0.2		76.8	24.2	0.4			
	Early	67.3	32.7			98.9	1.1				
Mixture	Medium	745	25.5			07.9	2.2				
	early	/4.5	23.3			97.8	2.2				
	Evennes	s index		0.507	0.683			0.589	0.724		

Table 3. Botanical composition of I-II productive re-growths in the sowing year (%)

## Conclusions

In the year of sowing, the development and growth rate, and biomass productivity, depend first on the species and then on cultivar. In pure stands Naki, Bajka and Diament of *L. perenne* and Wanda of *F. pratensis* reveal good development and forage productivity. The development of *D. glomerata*, *Ph. pratense* and *P. pratensis* cvs in mixtures is evidently slower than in pure stands. The mixtures developed and yielded similarly, but the yields were much lower compared to their *L. perenne* components in pure stands – Naki and Bajka cvs, especially in the second re-growth.

## References

De Vliegher A. and Carlier L. (2002) Grass seed mixtures of species and varieties: a higher value for agricultural use than a single variety? 24<sup>th</sup> EUCARPIA Fodder Crops and Amenity Grasses Section Meeting, 70–74. Kirwan L. *et al.* (2007) Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology* 95, 530–539.

Rutkowska B. and Janicka M. (1992) Biomass formation of five grass species in the seeding year. Annals of Warsaw Agricultural University – SGGW, Agriculture 24, 45–52.

Rutkowska B., Janicka M. and Lewicka E. (1994) Development and growth of grass and legume cultivars in the year of sowing in pure stands and meadow mixtures depending on the level of nitrogen fertilization. *Genetica Polonica* 35A, 291–298 (in Polish with English summary).

Vinczeffy I. (1984) The effects of some ecological factors on grass yield. *Proceedings of the 10<sup>th</sup> General Meeting of European Grassland Federation*, Ås, Norway, 76–79.

# Yield of feed units obtained from orchard grass cultivated as monoculture and in mixtures with legume plants

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## Abstract

Field investigations were carried out in 2002–2004. The objective was to compare orchard grass (cocksfoot) cultivated as monoculture (100%) and in two mixtures: orchard grass 50% + alfalfa 50%, and orchard grass 50% + red clover 50%. Tested plants were harvested three times per year. They were fertilized at four different levels of nitrogen (0, 30, 60, 90 kg N ha<sup>-1</sup>). The metabolizable energy in fodder and digestible protein content were estimated. In comparison with pure orchard grass, herbage of the orchard grass-legume mixtures had higher contents of digestible protein, more metabolizable energy and larger quantities of fodder units in the dry matter. However, averaged across the range of N inputs, the highest yields of feed units were obtained from the orchard grass monoculture swards.

Keywords: orchard grass, red clover, alfalfa, metabolic energy, feed unit

## Introduction

Supplementation of natural grasslands in ruminant nutrition is often based on grasses and grass-legume mixtures established on arable land. Of the species used under field conditions, orchard grass (cocksfoot: *Dactylis glomerata* L) and legumes such as alfalfa (lucerne: *Medicago sativa*) or red clover (*Trifolium pratense* L.) are important forage species (Jodełka *et al.*, 2006). Satisfying the requirement for energy and protein for ruminants is an important requirement. There is also a need to obtain proportions of individual components in mixtures that maintain legumes in the sward. The need to apply the correct amount of nitrogen fertilization is also an important concern. In assessing the suitability of these forage plants for the production of roughage, special attention needs to be given to their energy and protein content (Piecuch *et al.*, 1997). A significant excess or deficiency of energy or protein in the nutrition may lead to a reduction of feed conversion efficiency. This paper presents results of the feed evaluation of orchard grass, grown as monoculture and in mixture with alfalfa or red clover, in the context of protein and energy content of forages.

## Materials and methods

The study was conducted in 2002–2004. The field experiment was located on arable land. The soil is classified as arenosole type and is characterized by a slightly acidic reaction (pH in 1 N KCl is 5.12), low contents of total nitrogen, magnesium, copper and zinc, a very high content of phosphorus, and an average content of potassium. The experiment was established in spring 2002 with three replications in a split-plot design with an area of 10 m<sup>2</sup>. The treatments were orchard grass as monoculture and mixtures of orchard grass with alfalfa and red clover, grown as follows: (i) orchard grass cv. Reda – 100%, (OG 100%); (ii) orchard grass cv. Reda – 50% + alfalfa cv. Legend – 50%, (OG 50% + A 50%); (iii) orchard grass cv. Reda – 50% + red clover cv. Nike – 50% (OG 50% + 50% RC).

In the experiment four levels of nitrogen fertilization (0, 30, 60, 90 kg N ha<sup>-1</sup>) were used. Potassium was applied at 100 kg  $K_2O$  ha<sup>-1</sup>. Phosphorus was not applied due to the high

content of this element in the soil (48 mg  $P_2O_5$  in 100 g of soil). Nitrogen as ammonium nitrate, and potassium as 60% potassium salt, were used each study year in three equal doses (one for each regrowth).

In each season, three cuts were taken. Immediately after cutting, the fodder from each plot was weighed and 0.5 kg sample of green mass collected for chemical analysis. On the basis of crude protein, crude fibre and crude fat content, the amount of metabolizable energy in the feed was calculated by the DLG system – dietary doses of the German system (Zarudzki *et al.*, 1997). The quantity of feed contained in units of plant matter was calculated according to the pattern given by Domański (1987). The results were statistically analysed using the two factors in a split-plot design. The significance of differences between means characterizing the studied factors was estimated using the Tukey's test at the significance level P < 0.05.

### Results

Analysing the impact of nitrogen fertilization on metabolic energy content (Table 1) in different combinations showed that orchard grass fertilized with nitrogen accumulated significantly more energy than that grown in the nil-N control plots. However, the increase of N dose did not always result in an increase in energy content for this species.

Combination		Dose 1	N kg ha <sup>-1</sup>		Mean
Combination	0	30	60	90	8.17
OG	8.06	8.15	8.28	8.20	8.24
OG 50% + A 50%	8.23	8.23	8.26	8.25	8.38
OG 50% + RC 50%	8.39	8.40	8.37	8.37	8.26
Mean	8.23	8.26	8.30	8.27	
$LSD_{0.05} \leq \text{for}$					
Fertilization (A)	0.02				
Combinations (B)	0.13				

Table 1. Metabolizable energy content in orchard grass and in orchard grass-legume mixtures (MJ  $kg^{-1}DM$ ) depending on the dose of nitrogen (average from 2002–2004)

The content of feed units (Table 2) in monoculture of orchard grass and its mixtures with legume plants was similar.

Table 2. The content of feed units in orchard grass and in mixtures (JP kg<sup>-1</sup> DM) depending on the nitrogen dose (average from 2002–2004)

Combination		Dose	N kg ha <sup>-1</sup>		Mean	
Comoniation	0	30	60	90	Iviean	
OG	1.12	1.14	1.17	1.17	1.15	
OG 50% + A 50%	1.15	1.16	1.18	1.19	1.17	
OG 50% + RC 50%	1.16	1.15	1.17	1.19	1.17	
Mean	1.15	1.15	1.17	1.18		
$LSD_{0.05} \leq \text{for}$						
Fertilization (A)	0.02					
Combinations (B)	0.01					
Interaction (AxB)	0.02					

However, statistical analysis of these results showed significantly more of these units in mixtures than in orchard grass monoculture (Table 2).

Differences in the amounts of feed units between the nitrogen doses (Table 3) also occurred. The average content of feed units in plants fertilized with 90 kg N ha<sup>-1</sup> was the highest and

amounted to  $1.18 \text{ JP kg}^{-1} \text{ DM}$ . Moreover, fertilization at 90 kg N ha<sup>-1</sup> significantly increased the number of feed units relative to the control and the 30 kg N ha<sup>-1</sup> treatments, in both mixtures and monoculture of orchard grass.

Combination		Dose 1	N kg ha⁻¹		— Mean	
Combination	0	30	60	90	Mean	
OG	4320	5839	8363	8380	6725	
OG 50% + A 50%	4286	5381	6032	7237	5734	
OG 50% + RC 50%	4355	5163	6037	7220	5694	
Mean	4320	5461	6811	7612		
LSD $_{0.05} \leq \text{for}$						
Fertilization (A)	780					
Combinations (B)	991					
Interaction (AxB)	640					

Table 3. Yield of feed units (JP  $kg^{-1}$  DM) in orchard grass and in mixtures in the depend on the nitrogen dose (average from 2002–2004)

### Conclusions

In comparison with orchard grass monoculture, the tested mixtures were characterized by forage with lower contents of feed units and a higher yield of feed units. Forage from the mixture of orchard grass with red clover had the highest metabolizable energy content. The energy value of orchard grass and the mixture with alfalfa were similar.

The rates of nitrogen application used in the experiment resulted in differences in the values of the tested parameters.

The mixture of orchard grass with red clover, compared with the control treatment, provided the highest metabolizable energy at fertilization with 30 kg N ha<sup>-1</sup>. The yield of feed units of orchard grass as pure sward, and in mixtures with legumes, increased with increased rates of fertilizer nitrogen.

## References

Domański P. (1987) System of tests and the criteria and methods for assessing the economic value of forage grass varieties in Poland. *Wiadomości Odmianoznawcze* 22 (2–3), 26–33 [in Polish].

Jodełka J., Jankowski K., Mateńko M. and Ciepiela G.A. (2006) Usefulness of red clover and alfalfa for the cultivation with orchard grass on the arenosol types of soils. *Ląkarstwo w Polsce* 9, 79–86 [in Polish]. Piecuch A., Krzywiecki S. and Szyszkowska A. (1997). The energy value of pasture grass sward and grass-clover determined by the NEL and Nir '88<sup>th</sup> method. *Biuletyn Oceny Odmian* 29, 185–189 [in Polish]. Zarudzki R., Traczykowski A. and Mroczko L. (1997) *DLG – tables of food values and norms of ruminant feed nutrition*. Wyd. Przedsiębiorstwo Produkcyjno-Handlowe VIT-TRA, 272–275 [in Polish].

# Seedling recruitment of *Trifolium repens* in grass-clover swards is affected by grassland management and season

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### Abstract

The population structure of *Trifolium repens* was recorded in swards which, for four preceding years, had been fertilized (0, 10 or 30 g N m<sup>-2</sup> yr<sup>-1</sup>) and harvested differently (two or four cuts per season) and where *Poa pratensis* was the initial companion species. The clonal developmental stages were dominating in all *T. repens* populations, irrespective of level of nitrogen supply, cutting frequency and season. However, there were seedlings in all sward types, with the largest proportion of the population in heavily fertilized and seldom-cut plots with tall vegetation, a high proportion of bare ground and low abundance of *T. repens*. Seedling recruitment can therefore be an important mechanism maintaining the population in time, especially when the population size is small.

Keywords: Trifolium repens, population structure, management, seedlings, fragments

## Introduction

Survival and maintenance of *T. repens* population in mixed grass-clover swards depend on clonal growth, including phytomer formation and recruitment from seed. In established populations of clonal plants, clonal fragments are dominant and seedling recruitment is often rare (Cook, 1985; Eriksson, 1989). Seedlings are, however, important for establishing populations in new habitats and maintaining genetic variation in the population (Eriksson, 1997). The hypothesis tested in the present study was that the abundance of *T. repens* in mixed swards is related to its population structure and winter mortality of population members.

## Materials and methods

The study was located in central Norway, in an eight-year-old pasture dominated by *T. repens* and *Poa pratensis*. In 2001 a factorial experiment with 24 plots of  $4\times4$  meter was established including cutting frequency (twice (C2) or four times C4) and nitrogen supplies. Nitrogen was supplied from a compound mineral fertilizer (N-P-K, 18:3:15) at rates of 0 (N0), 10 (N10) or 30 (N30) g m<sup>-2</sup> per year.

*T. repens* plants were sampled in half of the plots in autumn 2004 and in the remaining plots in early spring 2005. Sampled plants were classified into one of four developmental stages: seedling, juvenile taprooted (derived from seedlings), mature taprooted, and fragments (without taproot). Cover of bare ground in the plots was determined by image analysis. Digital photos taken at 80 cm height ten days after the last cut in 2004 were processed using software especially developed for the purpose, as explained in Bonesmo *et al.* (2004). Prior to the last cut in autumn 2004, the vegetation height was measured in all plots, by the use of a rising plate meter (Mould, 1992). Sixty four independent recordings

were taken in each plot. The abundance of *T. repens* was highest in plots not fertilized and cut four times and lowest in plots cut twice and fertilized.

Difference in frequency of each developmental stage within a plot was tested in a log linear model (GLM with poisson errors). To test the effect of cutting frequency, nitrogen fertilization and time, saturated and reduced models were compared in a hierarchical manner, and non-significant terms were excluded from the model step-wise (Quinn and Keough, 2002). ANOVA was used to test the effect of cutting regime and nitrogen supply on vegetation height and proportion of bare ground.

### Results

There were three-way interaction effects among developmental stage, cutting and time (df = 15, P = 0.005) as well as developmental stage, cutting and nitrogen (df = 18, P < 0.001) in the frequency of plants. There were interaction effects between cutting and time (Figure 1a), and between cutting and nitrogen (Figure 1b) affecting the stage distribution of plants. Fragment was the most frequent developmental stage in all plots. Seedling and juvenile tap-rooted plants were most frequent in plots with the C2 and N30 (Figure 1b) combination, and in C2 plots in the autumn sampling (Figure 1a). There were seedlings in all treatments.

The vegetation height previous to the last cut was largest in C2 plots ( $F_{1, 18} = 96.28$ , P < 0.001, mean and sd: C4  $9.53\pm1.84$  cm, C2  $16.54\pm1.47$  cm). The proportion of bare ground was also highest in C2 plots ( $F_{1, 18} = 62.36$ , P < 0.001, mean and sd: C4  $0.24\pm0.07$ , C2  $0.63\pm0.18$ ,). Vegetation height and proportion of bare ground was not different among the different levels of nitrogen supply.

### Discussion

The fragments developmental stage was the dominant stage in all T. repens populations, which is typical of clonal species (Eriksson, 1997). The high frequency of fragments probably reflected that all the experimental swards were old and at advanced stages of development and, further, that seedling recruitment and survival were low. Although the frequency of plants at juvenile stages generally was low, there were differences between treatments types. The proportion of seedlings and juvenile tap-rooted plants were highest in plots with less frequent cutting and highest rate of nitrogen supply (C2 N30), i.e. with low abundance of T. repens. C2 plots had more bare ground suitable for seedling establishment, compared to plots with shorter vegetation and higher density of stolon and grass tillers at soil surface and consequently more competition for space. The results indicate that seedlings had high winter mortality, especially in C2 plots. There were seedling and juvenile tap-rooted plants present in all treatments. This is in contrast to previous conclusions that seedling recruitment is low or absent in grass-white clover swards (Chapman, 1987; Ennos, 1981). Seed production and seedling establishment may therefore have an important impact on T. repens population dynamics and persistence. The highest proportion of seedlings was in plots with low abundance of white clover. The high proportion of seedling recruitment in these plots could have been an important mechanism for maintaining the population over time. By changes in fertilization or management regime (e.g. reduced nitrogen fertilization and increased cutting frequency) these seedlings can provide a basis for population increases.



Figure 1. Mean and standard error of proportion of *T. repens* plants among four developmental stages with combination of sampling time and cutting regimes C2 and C4 (two and four cuts per season) (A) and of nitrogen supply levels N0, N10, N30 (0, 10 and 30 g m<sup>-2</sup>yr<sup>-1</sup>) and cutting regimes (B). Total number of plants in the experiment within the stages seedling, juvenile taprooted, mature taprooted and fragment was 90, 51, 58 and 898

### References

Bonesmo H., Kaspersen K. and Bakken A.K. (2004) Evaluating an image analysis system for mapping white clover pastures. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science* 54, 76–82.

Chapman D.F. (1987) Natural re-seedling and Trifolium-repens demography in grazed hill pastures. 2. Seedling appearance and survival. *Journal of Applied Ecology* 24, 1037–1043.

Cook R.E. (1985) Growth and development in clonal plant populations. Population biology and evolution of clonal organisms, Jackson J.B.C., Buss L.W. and Cook R.E. (eds), Yale University Press, New Haven and London, pp. 259–296.

Ennos R. (1981) Detection of selection in populations of white clover (*Trifolium repens* L). *Biological Journal of the Linnean Society* 15, 75–82.

Eriksson O. (1989) Seedling dynamics and life histories in clonal plants. Oikos 55, 231-238.

Eriksson O. (1997) Clonal life histories and the evolution of seed recruitment. *The ecology and evolution of clonal plants*, De Kroon H. and van Groenendael J. (eds), Backhuys Publishers, Leiden, pp. 221–226.

Mould F.L. (1992) Use of a modified rising-plate meter to assess changes in sward height and structure. *Norwegian Journal of Agricultural Sciences* 6, 375–382.

Quinn G.P. and Keough M.J. (2002) *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge.

# The impact of precipitation on yield of *Dactylis glomerata*, *Dactylis polygama*, *Festuca arundinacea* and genus hybrids in 1986–2011

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## Abstract

At the Jevíčko site in the Czech Republic we evaluated production ability and yield variability of five varieties of *Dactylis glomerata* L. (DG), one variety of *Dactylis polygama* (DP), two varieties of *Festuca arundinacea* L. (FA) and two intergenus hybrids. The trials were established by grassland renovation in 1986 at Jevíčko (343 m a.s.l., mean annual temperature 7.4°C, average annual precipitation 545 mm). Grass species were sown as simple legume-grass mixtures with red clover and white clover and fertilised with 240 kg ha<sup>-1</sup> N+P<sub>35</sub>K<sub>100</sub>, and utilised in three cuts. This contribution evaluates dry matter production, botanical composition of stand and yield variability. *Dactylis glomerata*, *Festuca arundinacea* and intergenus hybrids are persistent and reached high dry matter production. The yield in individual years was significantly influenced more by total rainfall in vegetation season than the total annual precipitation.

Keywords: grasses, production ability, yield variability, *Dactylis glomerata*, *Dactylis polygama*, *Festuca arundinacea*, festulolium hybrid

## Introduction

In the last two decades the frequency of occurrence of dry years has been increasing due to a global climate change and has had a significant impact on permanent grassland production. Yield variability can be evaluated on the basis of long-term trials with perennial species. The use of Festuloliums has increased in advanced forage countries in the last two decades due to their agricultural properties such as production ability, persistence, etc. (Grønbæk, 2010). DG, FA and festucoid hybrids belong among the most productive and persistent grasses in the Czech assortment of grasses (Kohoutek *et al.*, 2004). Selection of ecologically compatible grass and clover species for grassland renovation is an objective of a long-term research in the Czech Republic.

## Material and methods

Nineteen grass species and varieties were sown at the Jevíčko site in 1986 in order to evaluate production and persistence of *Dactylis glomerata*, [three varieties (Niva, Rela, Milona) and two newly bred varieties VV-24/75, VV-126/81 (cv. Dana, 1992)], *Dactylis polygama* [(syn. *Dactylis Aschersoniana* Graeb.), since 1998 Tosca], *Festuca arundinacea* L. [one variety (Lekora) and one newly bred variety HŽ-BI (Kora, 1989)] and genus festucoid hybrids [two newly bred varieties MRK-HŽ I (Felina, 1988), MRK-HŽ III/3 (Mahulena, registered in Germany in 2010)]. The experimental site is located in moderately warm and moderately wet region with altitude 343 m a.s.l., annual mean temperature 7.4°C and annual average precipitation 545 mm, a long-term average precipitation in vegetation season (1 April – 30 September) was 347 mm (Tolasz *et al.*, 2007) given as the average for the years between 1966–1995. Soil type was classified as fluvisol. Grasses were sown at the Jevíčko site in 19 simple grass-legume mixtures (sowing mixture composition was: 60% grass species, 25% *Trifolium pratense* L., 15% *Trifolium repens* L.). These were studied in a 3-cut system.

Grass-legume mixtures and pure cultures were fertilised with nitrogen at the rate of 120 kg ha<sup>-1</sup> (1987), 180 kg ha<sup>-1</sup> (1988 and 1989) and 240 kg ha<sup>-1</sup> (1990–2011). The acquired findings were evaluated with linear regression, correlation coefficients (r) were calculated and critical values  $r_{\alpha}$  of correlation coefficient for  $\alpha_{0.05}$  and  $\alpha_{0.01}$  were determined. Standard deviation (s, resp. SE) and average mean error ( $s_{\overline{x}}$ , resp. SEM) for dry matter production.

Standard deviation 
$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \overline{x})^2}{n-1}}$$
; average mean error  $(s_{\overline{x}} = \frac{s}{\sqrt{n}})$ 

#### **Results and discussion**

Annual dry matter production during 1986-2011 (Table 2, Figure 1) of the evaluated species was as follows: DG 11.49 t DM ha<sup>-1</sup>, s = 2.96 t ha<sup>-1</sup>; DP 10.70 t ha<sup>-1</sup>, s = 2.62 t ha<sup>-1</sup>; FA 11.91 t ha<sup>-1</sup>, s = 3.34 t ha<sup>-1</sup>; and FlH 11.90 t ha<sup>-1</sup>, s = 3.11. This proved high production abilities of DG, FA and FlH (Kohoutek *et al.*, 2004). Dry matter production is more influenced by total rainfall in the vegetation season than by total annual rainfall (Table 1). The linear equation demonstrates that 100 mm annual rainfall at the site increases mean yield by 0.41 t ha<sup>-1</sup> (FlH by 0.58 t ha<sup>-1</sup>, DP by 0.51 t ha<sup>-1</sup>, FA by 0.30 t ha<sup>-1</sup>, DG by 0.23 t ha<sup>-1</sup>) but all results were inconclusive (ns). In the vegetation season 100 mm rainfall increases yield in the average of species by 1.16 t ha<sup>-1</sup> (P = 0.01): DP by 1.19 t ha<sup>-1</sup> (P = 0.05), FlH by 1.39 t ha<sup>-1</sup> (ns), FA 1.11 t ha<sup>-1</sup> (ns) and DG by 0.97 t ha<sup>-1</sup> (ns).

			Equati	on parameters	<ul> <li>Correlation</li> </ul>					
Species	No. of	n	а	b	coefficient					
	varieties		(t ha <sup>-1</sup> )	$(t ha^{-1} mm^{-1})$	(r)					
Yield dependence on total annual precipitation (mm)										
Dactylis glomerata L.	5	25	10.20	0.0023	0.06 (ns)					
Dactylis polygama	1	25	7.89	0.0051	0.16 (ns)					
<i>Festuca arundinacea</i> L.	2	25	10.21	0.0030	0.08 (ns)					
Festulolium (festucoid)	2	25	8.61	0.0058	0.16 (ns)					
Collection of all species		100	9.23	0.0041	0.11 (ns)					
Yield de	ependence of	n vegeta	ation season	rainfall (mm)						
Dactylis glomerata L.	5	26	7.97	0.0097	0.27 (ns)					
Dactylis polygama	1	26	6.36	0.0119	0.38*					
Festuca arundinacea L.	2	26	7.87	0.0111	0.27 (ns)					
Festulolium (festucoid)	2	26	6.85	0.0139	0.36 (n s)					
Collection of all species		104	7.26	0.0116	0.34**					

Table 1. Parameters of linear equation y = a+bx characterizing DM production of evaluated grass species depending on total annual precipitation and rainfall in the vegetation season

Statistically significant (P < 0.05 = \*; P < 0.01 = \*\*), ns = statistically non-significant.

Table 2. Dry	<sup>r</sup> matter	production,	standard	deviation	(s) a	ind a	iverage	mean	error	$(s_{\overline{x}})$	of	four	grass
species in av	erage of	f the period	1986-201	1									

		Dry matter production	
Species	DM	S	$S_{\overline{x}}$
-		(t ha <sup>-1</sup> )	
Dactylis glomerata L.	11.49	2.96	0.58
Dactylis polygama	10.70	2.62	0.51
<i>Festuca arundinacea</i> L.	11.91	3.34	0.65
Festulolium (festucoid)	11.90	3.11	0.61



Figure 1. Impact of total annual precipitation (1986–2010, n = 100) and of vegetation season rainfall (1986–2011, n = 104) on dry matter production of evaluated species

The presented long-term results will be further analysed by a growth model, including more independent environmental factors, from the viewpoint of impact on yield using the procedure of Trnka *et al.* (2011).

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### References

Grønbæk O. (2010) Festulolium in Scandinavia. Combining yield, forage quality and persistence. In: Kohoutek A. (ed.), *Kvalita píce z travních porostů a chov skotu v měnících se ekonomických podmínkách*. VÚRV, v.v.i., Praha, pp. 5–8.

Kohoutek A., Odstrčilová V., Komárek P. and Nerušil P. (2004) Persistence and production ability of *Dac*tylis glomerata L., *Dactylis polygama* Horvat, *Festuca arundinacea* L. and genus hybrids in 1986–2003. *Grassland Science in Europe* 9, 422–424.

Tolasz R., Brázdil R., Bulíř O., Dobrovolný P., Dubrovský M., Hájková L., Halásová O., Hostýnek J., Janouch M., Kohut M., Krška K., Křivancová S., Květoň V., Lepka Z., Lipina P., Macková J., Metelka L., Míková T., Mrkvica Z., Možný M., Nekovář J., Němec L., Pokorný J., Reitschläger J.D., Richterová D., Rožnovský J., Řepka M., Semerádová D., Sosna V., Stříž M., Šercl P., Škáchová H., Štěpánek P., Štěpánková P., Trnka M., Valeriánová A., Valter J., Vaníček K., Vavruška F., Voženílek V., Vráblík T., Vysoudil M., Zahradníček J., Zusková I., Žák M. and Žalud Z. (2007) Atlas podnebí Česka–Climate Atlas of Czechia. 1<sup>st</sup> edition, ČHMÚ Praha, Olomouc, 255 pp.

Trnka M., Bartošová L., Schaumberger A., Ruget F., Eitzinger J., Formayer H., Seguin B. and Olesen J.E. (2011) Climate change and impact on European grasslands – review. *Grassland Science in Europe* 16, 39–51.

# Occurrence of herbs in intensively used meadow communities constituting part of the Natura 2000 Network

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## Abstract

The aim of the investigations was to determine the impact of meadow and pasture utilisation, as well as site conditions, on the occurrence of plant species commonly included in the utilisation group known as 'herbs'. On meadows mown 2–3 times during the vegetation season, as well as on meadows constituting part of the Natura 2000 network (single-cut), phytosociological relevés were made using the Braun-Blanquet method; floristic diversity of grass communities, their botanical structure, natural value and sward use-value were then assessed. The highest quantities of herbs could be found in extensively managed communities and, from among ecological factors, moisture site conditions, in particular, determined species numbers and their proportion in the swards.

Keywords: herbs, meadow communities, utilisation, Natura 2000

## Introduction

Site conditions as well as utilisation influence the biodiversity of meadow communities, especially the occurrence of dicotyledonous species. Kozłowski and Stypiński (1997) maintain that floristic diversity of grass communities in Poland is affected by 350 plant species. These plants comprise, among others, species improving feed palatability and frequently exhibiting therapeutic properties (Trzaskoś, 1996; Trąba and Wyłupek, 1999). Multispecies swards of extensively utilised communities can even exceed the fodder value of meadows considered as the most valuable in this respect. On the other hand, intensively utilised swards provide increased yields and fodder value but, at the same time, result in simplification of the species composition of plant communities (Grzegorczyk and Alberski, 2004). Reduction of the presence of herbs in the sward can lead to deterioration in animal welfare. On the other hand, many species of dicotyledonous plants can, in favourable conditions, significantly increase their proportions in the sward and replace other valuable species, e.g. grasses and legumes, causing reduction of fodder value. The objective of the study was a comparative analysis of the occurrence of dicotyledonous plant species from the group of herbs in the sward of meadow communities differing with respect to site conditions and utilisation intensity. In addition, the impact of herbs on the sward fodder value score and natural quality of meadow was also evaluated.

## Materials and methods

Herb occurrence in meadow communities, i.e. plant species characterised by medicinal or flavour properties, or properties for improving sward fodder value, was assessed on the basis of analyses of phytosociological reléves taken with the assistance of the Braun-Blanquet method in the period May-August from experimental plots of 75–100 m<sup>2</sup> area. The analysed swards were derived from intensively managed communities (Table 1) as well as from those cut only once a year which constituted part of the Natura 2000 network.

The following parameters were determined in the examined communities: mean species number (including herbs) in phytosociological relevés, their proportion (expressed as percent

of the occupied area) and frequency of occurrence of some of them, and possibilities of their application (Kota *et al.*, 1998). Natural values were assessed on the basis of Oświt's (2000) valorisation number, whereas sward fodder value score (FVS) was estimated according to Filipek (1973). Site conditions of the examined plant communities were evaluated using Ellenberg's (1992) bioindicator method.

Table	1.	Utilisation	frequency	of inter	nsively	utilised	sward	communities
			1 2		2			

Utilisation	Community
$2 \times cut$	Caricetum acutiformis, Molinietum caeruleae, com. Deschampsia caespito-
$3 \times cut$	sa, Caricetum gracilis, com. Agrostis stolonifera, Holcetum lanati Phalaridetum arundinaceae
J × Cut	1 naturtaetum aranamaceae

### **Results and discussion**

The examined meadow communities were characterised by considerable variations in their site conditions, which affected their floristic composition as well as proportions of herb plant species. From among the site elements, moisture was found to exert a strong influence on species abundance of meadow communities and, consequently, also on the occurrence of herbs. Declining site moisture of plant communities from the *Phragmitetea* class (*Phala-ridetum arundinaceae, Caricetum acutiformis* and *C. gracilis*) facilitated intensive sward utilisation and contributed most frequently to increased species numbers, although, at the same time, it contributed to distinct reduction of areas occupied by herbs (Table 2).

On the other hand, in the case of communities of the *Molinio-Arrhenatheretea* class, greater species numbers were observed most frequently in swards developed in sites of lower moisture (F from 5 to 6) and lower content of soil nitrogen (N from 3 to 4.5) and which were cut only once during the season. In such conditions, the proportion of herbs in the area of phytosociological reléves was smaller.

		E	llenber	g's inde	Num	ber of	Herbs	plant			
Plant community Plass: Phragmitetea Phalaridetum arundinaceae Caricetum acutiformis Caricetum gracilis Class: Molinio-Arhenatheretea With Deschampsia caespitoso With Agrostis stolonifera	E		р		N	N		plant species/		ight	
Plant community		Г		К		1		relevé		percent)	
	E*	I*	E	Ι	Е	Ι	E	Ι	Е	Ι	
Class: Phragmitetea											
Phalaridetum arundinaceae	8.5	7.6	5.2	5.2	5.9	5.8	21.2	16.5	16.0	5.0	
Caricetum acutiformis	8.0	6.5	5.2	3.6	5.1	4.7	17.7	24.3	24.1	18.9	
Caricetum gracilis	7.8	7.5	4.4	4.8	5.0	4.3	14.1	18.1	33.2	13.0	
Class: Molinio-Arhenatheretea											
With Deschampsia caespitosa	6.4	6.5	1.6	1.3	3.1	4.0	21.9	20.0	15.5	15.3	
With Agrostis stolonifera	5.5	4.2	2.4	1.8	5.2	4.9	23.5	21.4	41.0	34.6	
Holcetum lanati	5.3	5.4	2.0	2.0	4.2	7.8	30.9	17.2	14.2	28.2	

Table 2. Impact of site conditions	on proportions	of herb species
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<sup>1</sup> Ellenberg's index (1992): F – soil moisture, R – soil reaction, N– nitrogen content in soil. \*E – extensive utilisation (NATURA 2000 Network), \*I – intensive utilization.

\*E - extensive utilisation (NATURA 2000 Network), \*I - intensive utilization.

The swards of intensively utilised communities were characterised by the occurrence of greater proportions of species from the *Poaceae* family which contributed to improvement of their fodder value, whilst the swards of one-cut meadows comprised higher proportions of legumes as well as other dicotyledonous plants (including herbs). This structure of functional groups in extensively utilised meadow communities reduced sward fodder value score but exerted a positive influence on the significance of its natural value indicator (Table 3). The dicotyledonous plants most frequently occurring in the examined sward communities comprised: *Achillea millefolium, Taraxacum officinale, Plantago lanceolata, P. major* as

well as *Potentilla anserina*, *Daucus carota*, *Heracleum sphondylium* and *Sanguisorbia officinalis*. Many of these species are characterised by a favourable chemical composition which, apart from advantageous impact on fodder palatability, can also be utilised in cosmetic or pharmaceutical industries or as spices.

1	Functional groups (weight percent):						DVC**		Valorisa-		
gra	sses	sed	ges	legı	ıme	other	herbs	Г٧	2	tion i	ndex
E*	I*	Е	Ι	Е	Ι	Е	Ι	Е	Ι	Е	Ι
73.3	74.8	1.4	8.1	1.4	1.4	23.9	15.7	5.2	5.8	2.42	2.80
5.9	32.7	60.2	39.5	7.1	0.8	26.8	27.0	1.6	3.0	3.18	3.16
21.4	26.2	43.3	57.3	0.6	0.4	34.7	16.1	2.4	2.48	3.03	3.38
tea											
a 74.6	73.8	2.8	2.1	1.0	0.5	21.6	23.6	3.5	3.9	2.48	2.69
19.8	21.4	26.7	25.6	4.3	5.2	49.2	47.8	2.7	3.8	3.06	1.61
79.2	65.2	0.1	2.5	0.1	0.8	20.6	31.5	6.0	5.4	2.00	2.11
	$     \frac{I}{gras} \\     \hline         {} \frac{gras}{E^*} \\         73.3 \\         5.9 \\         21.4 \\         tea \\         a 74.6 \\         19.8 \\         79.2     $	Functi           grasses           E*         I*           73.3         74.8           5.9         32.7           21.4         26.2           tea         3           a         74.6         73.8           19.8         21.4           79.2         65.2	Functional g           grasses         sed           E*         I*         E           73.3         74.8         1.4           5.9         32.7         60.2           21.4         26.2         43.3           tea	Functional groups           grasses         sedges           E*         I*         E         I           73.3         74.8         1.4         8.1           5.9         32.7         60.2         39.5           21.4         26.2         43.3         57.3           tea	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$

Table 3. Structure of sward functional groups in the examined meadow communities vs. their fodder and natural values

\*E – Natura 2000, mowing meadows, 1 cut in year, \*I – intensive utilisation, 2–3 cut in year, \*\*FVS – fodder value score according to Filipek (1973).

As indicated by many researchers, changing habitat conditions as well as increased utilisation intensity exert influence on transformations in the floristic composition of plant communities (Nösberger and Kessler, 1997). Increased utilisation intensity, in particular, contributes to considerable decline in proportions of dicotyledonous species, which is sometimes also connected with domination by some of them. This can have a disadvantageous impact both on fodder palatability and sward natural quality. Both Hopkins *et al.* (1995) and Zarzycki *et al.* (2005) emphasise the need for the multispecies sward reconstruction of meadow communities because of the importance of this group of plants in animal nutrition.

### Conclusions

Extensive utilisation of areas included in the Natura 2000 Network favoured the occurrence of herbs as confirmed by their high numbers and great proportions in the sward. Sward of plant communities containing high proportions of herb species was characterised by low use-value but high natural values.

### References

Grzegorczyk S. and Alberski J. (2004) Content of nutrients in selected herb species on natural meadows. *Grassland Science in Europe* 9, 921–923.

Hopkins A., Pywell R. and Peel S. (1995) Restoration of botanical diversity of grassland by different methods of seed and plant introduction. *Annales Universitatis Mariae Curie-Sklodowska* Sectio E, Agricutura 24, 33–37.

Kota M., Kovacs B., Szilagyi Sz. and Vinczeffy I. (1998) The fodder value and mineral content of medicinal plants in grassland. *Grassland Science in Europe* 3, 433–436.

Kozłowski S. and Stypiński P. (1997) The grassland in Poland in the past, present and future. *Grassland Science in Europe* 2, 19–29.

Nösberger J. and Kessler W. (1997) Utilisation of grassland for biodiversity. *Grassland Science in Europe* 2, 33–42. Oświt J. (2000) *Marsh natural valorisation method and results of its application to selected objects*. Published by IMUZ in Falenty, 37 pp. [in Polish].

Traba C. and Wyłupek T. (1999) Therapeutic plants in the some meadow communities of Por river valley. *Folia* Universitatis Agriculturae Stetinensis 197, Agricultura 75, 321–324 [in Polish with English summary].

Trzaskoś M. (1996) Floristic, fodder and landscape values of of herbal meadows. Zeszyty Problemowe Postępów Nauk Rolniczych 442, 417–430 [in Polish with English summary].

Zarzycki J., Gałka A. and Górna-Drożdź E. (2005) Fodder value of the Pieniny National Park meadows sward. *Acta Scientiarum Polonorum*, Agricultura 4(2), 119–132 [in Polish with English summary].

## Persistency of selected grass species in meadow sward in a post-boggy habitat

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## Abstract

The aim of this research was to estimate the persistency of selected grass species in a meadow sward in a post-boggy habitat. The studies were carried out in 1996–2011 in a peatland complex in Sosnowica (Wieprz-Krzna Channel area in eastern Poland) on peat-muck soil (Mt II). The research included assessments of the share of various grass and legumes species. During the 15 years of the experiment, the species composition of the meadow sward changed considerably along with the changing hydrology. After 3 years, legumes and *Phleum pratense* disappeared from the sward, and after 5 years the proportion of *Festuca arundinacea* increased. The proportion of *Dactylis glomerata* was stable during the experiment. *Lolium perenne* also occupied a high proportion with a high level of persistency that was determined by the winter temperatures. The highest level of persistency during the study was exhibited by *Lolium perenne* and *Festuca arundinacea*, which ensured the continued diversity of the species composition and prevented *Poa pratensis* ssp. *angustifolia* from dominating.

Keywords: meadow, persistency, post-boggy habitat

## Introduction

Post-boggy habitats are characterised by considerable changes in the physico-chemical properties of the soil and flora. After several years of use involving cutting, the sward frequently becomes dominated by *Poa pratensis* ssp. *angustifolia* and dicotyledons, which considerably reduce the nutritional value of forage and the yield of grassland. Firstly, decreasing proportions of legumes and increase of grasses, such as *Festuca pratensis* and *Phleum pratense*, are observed (Bauer, 1981). The aim of this paper is to estimate the persistency of selected grass species in a meadow sward in a post-boggy habitat.

## Materials and methods

The studies were carried out in 1996–2011 in a peatland complex in Sosnowica (the Wieprz-Krzna Channel area in eastern Poland). The experiment was established in 1996 on a peatmuck soil (Mt II) characterised by an acidic pH (4.8 1m KCl) and low phosphorus and potassium content. Four seed mixtures were studied (Table 1).

Species (cultiver)	Mixtures						
Species (cultival)	А	В	С	D			
Lolium perenne (different cultivars)	0	30	0	35			
Festuca arundinacea (Rahela)	10	10	0	0			
Phleum pratense (Kaba)	50	20	31	20			
Dactylis glomerata (Areda)	10	10	15	10			
Trifolium repens (Romena)	15	15	27	17.5			
Trifolium pratense (Raba)	15	15	27	17.5			

Table 1. Species composition of sown mixtures (%)

The following amounts of fertiliser were applied in the study years: N 40, P 35 and K 100 kg ha<sup>-1</sup>. From 2003, nitrogen fertilisation was increased to 70 kg ha<sup>-1</sup>. The sward was cut

three times at optimum dates for such communities. Each year, representative samples were collected from the first regrowth in order to determine the species composition of the sward by means of botanical and weight analysis. Correlation between the groundwater level in the previous growing season and soil moisture index based on the proportion of the particular species according to Zarzycki *et al.* (2002) were calculated. Soil moisture in the study years varied considerably and depended on precipitation and groundwater levels. In spring, the groundwater level was high (-20 to -40 cm), but fell in the subsequent months to -70 cm, or even to -100 cm in the years with small amounts of precipitation. In the years with high precipitation, the groundwater level was also high in the summer (-25 to -35 cm).

### **Results and discussion**

During the 15 years of the experiment, the species composition of meadow sward shaped by sowing changed considerably along with the changing hydrology. The hydrologic conditions were found to have a significant impact on the soil moisture index for the vegetation studied according to Zarzycki *et al.* (2002). The groundwater level and precipitation in the second half of the growing season (July-October) in the previous year exerted a significant impact (Table 2). It was manifested mainly in the occurrence of wet-habitat species (*Glyceria fluitans, Phalaris arundinacea*) in the meadow sward.

Table 2. Correlation between hydrologic conditions in a previous growing season and soil moisture index

Characteristic (July to October in a previ-	Groundwater level	Amount of precipitation	Hydrothermal Sieliani- nov coefficient
Soil moisture index	0.5375*	0.5655*	0.5540*
(Zarzycki et al., 2002)	The critical value of	5*n = 0.5214 (Ramsey,	
2.98-3.82		1989)	

The species composition of the sward was characterised by low stability during the first period of use. The proportion of the sown species in the meadow sward was high (above 60%) in the years 1997–2000, particularly in mixtures with *Lolium perenne* (B and D). In the following years, the share of sown species decreased considerably, which was linked to the reduced proportion of legumes in the sward after 3 years of use. This period was also marked by unfavourable climatic conditions, as the growing seasons of 1997, 2001 and 2006 were characterised by high rainfall combined with high groundwater levels, which resulted in a reduced proportion of valuable grass species and the emergence of wet-habitat species. In the winter period of 2002–2003, very low temperatures (–23°C) and a thin snow cover were observed. In consequence, the sown grass species, particularly *Lolium perenne* and *Dactylis glomerata*, were frostburnt and their proportion in the sward in 2003 was low (5.4–32.0%). The small proportion of sown species and the lower turf density was the reason for increasing the N fertilisation in 2003, which led to a gradual increase in the proportion of sown species (60–75%) in 2005–2007 and a stable situation in 2008–2011 (Figure 1).

*Lolium perenne* was characterised by a stable proportion in the sward, except for the years 2002 and 2003. After the wet second half of the growing season in 2001 (groundwater level at -10 to -30 cm), the dry year of 2002 (280 mm of precipitation) and frosty winter of 2002–2003, the proportion of this species fell to 5.5%. The following years, however, saw an increase in the proportion of *Lolium perenne*, particularly in mixture D (35–45%). *Phleum pratense* had a high proportion in the years 1997–1998, particularly in mixtures C and D, while in the following years its proportion was considerably lower. The proportion of *Dactylis glomerata* was high in the years 1999–2001 (15.1–34.2%) and 2005–2007 (10.1–34.3%).



Figure 1. Changes in species composition of meadow sward in 1997-2011

It is worth noting the quite large but unstable proportion of *Festuca arundinacea*. Legumes turned out to be the least persistent. The sown species disappearing from the sward were replaced by *Poa pratensis*, whose proportion successively increased, particularly in A and C mixtures (Figure 1). Increasing proportions of *Phalaris arundinacea*, *Holcus lanatus* and *Deschampsia caespitosa* in the sward were observed

### Conclusions

Lolium perenne and Festuca arundinacea were characterised by the greatest persistence in the meadow sward in a post-boggy habitat. The persistence of these particular species was significantly influenced by hydrologic conditions, particularly precipitation and groundwater levels. Low temperatures in winter caused a decrease in the proportion of *Lolium perenne* and *Dactylis glomerata*. It should be stated that *Lolium perenne* characterised by ability to recover from damage caused by frost as well as to compete with *Poa pratensis* ssp. *angustifolia*. The lowest persistence was demonstrated by legumes, which only had a high proportion in the sward in the first 3 years of the experiment.

## References

Bauer U. (1981) Zur Leistungsdauer des Saatgraslandes 1 Mitt Saatgrasland für Weidenutzung. Arch. Acker-Pflanzenbar Bodenkd. 25(6), 373–380.

Ramsey P.H. (1989) Critical Values for Spearman's Rank Order Correlation. *Journal of Educational Statistics* 14(3), 245–253.

Zarzycki K., Trzcińska-Tacik H., Różański W., Szeląg Z., Wołek J. and Korzeniak U. (2002) Ecological indicator values of vascular plants of Poland. IB PAN Kraków, pp. 183.

# Effects of phosphorous and potassium fertilization on spring weed invasion in alfalfa meadows

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# Abstract

A field trial was conducted from 2005 to 2007 in north-eastern Italy to study the influence of phosphorous and potassium fertilization on the performance of alfalfa meadows. Alfalfa (*Medicago sativa* L.) cv. Delta was seeded on 30 March 2005 in a silty-loamy soil. Nine treatments deriving from the factorial combination of three rates of phosphorous (0, 100 and 200 kg ha<sup>-1</sup> year<sup>-1</sup> of  $P_2O_5$ ) and three rates of potassium (0, 300 and 600 kg ha<sup>-1</sup> year<sup>-1</sup> of  $K_2O$ ) were compared in a randomized complete block design. In order to study the effects of phosphorous and potassium fertilization on spring weed invasion, root dry weight (g plant<sup>-1</sup>), root diameter (mm) and stand density (plant m<sup>-2</sup>) at the last harvest of 2007, and the weed cover (%) at spring 2007, were then examined. Weed cover was not influenced by phosphorous rates, but decreased with increasing potassium rates. This result was likely due to the increase of plant vigor in consequence of potassium application as revealed by the increase of root dry weight, root diameter and individual plant yield.

Keywords: fertilization, phosphorous, potassium, weed, alfalfa

## Introduction

Both yield and forage quality of alfalfa can be severely reduced by weeds. Weed control in alfalfa mostly depends on cultural practices that directly influence stand density and plant vigor (Parrini and Bonari, 2002; Hall *et al.*, 2004). Nutrient management plays an important role to maintain a thick and vigorous stand especially when alfalfa is subjected to intensive harvest regime. The aim of this study was to provide information on the influence of phosphorous (P) and potassium (K) fertilization rates on spring weed invasion in alfalfa harvested at flower bud stage in north-eastern Italy.

## Materials and methods

A field trial was carried out for three years (2005–2007) at the Experimental Farm of Padova University in Legnaro (Padova) Italy (45°20'N, 11°58'E, elevation 8 m) to study the response of alfalfa to three rates each of P and K fertilizer. The fertilizer rates consisted of: 0, 100 and 200 kg ha<sup>-1</sup> year<sup>-1</sup> of  $P_2O_5$  and 0, 300 and 600 kg ha<sup>-1</sup> year<sup>-1</sup> of  $K_2O$ . The first fertilization was applied before seeding, and the subsequent fertilization was applied in December 2005 and 2006. Treatments were compared in a randomized complete block design with three replicates with plots size of 8 m<sup>2</sup> (6×5 m). Alfalfa cv. Delta was seeded on 30 March 2005 on a silty-loamy soil with pH of 8.15, and characterized by a high content of available phosphorous (31.5 mg kg<sup>-1</sup>) and a medium content of exchangeable potassium (145 mg kg<sup>-1</sup>). The climate of the site is a humid subtropical with an average annual temperature of 12.3°C and 820 mm of total rainfall. During the investigation period the monthly mean air temperatures ranged from 2.3 to 24.9°C, the lowest temperatures occurred on 24 and 25 January 2006

 $(-8^{\circ}C)$  while the highest on 19 July 2007 (36.9°C). Plots were cut at flower bud stage and received four harvests in 2005 and seven harvests in 2006 and 2007. Results of dry matter yield, forage quality and P and K concentrations in soil have been reported in a different work (Macolino *et al.*, submitted). The results regarding the percentage ground cover occupied by weeds (weed cover), stand density, root parameters and individual plant yield of the last growing season are reported here. Weed cover percentage was visually estimated in April 2007 and in October 2007, immediately after the last harvest, one meter of row in each plot was taken to determine stand density, and to measure root diameter at 2 cm below the crown by means of digital caliper. Subsequently, roots were cut at 15 cm below the crown and oven dried at 105°C for 36 h to determine dry weight. Individual plant yield of 2007 was calculated based on total annual yield and stand density. Data were subjected to the analysis of variance using SAS Proc Mixed (version 9.2; SAS institute, Cary, NC). Fisher's Protected LSD tests were used to identify significant differences between means. Correlations between root parameters, individual plant yield, stand density and weed cover percentage were also performed with the Graphpad software package.

#### **Results and discussion**

Results of analysis of variance for percentage weed cover showed significant difference among K rates, while the effect of P and interaction were not significant. The lack of difference in weed cover among P rates was probably due to the high P level in the soil at seeding.

Potassium application reduced weed cover, and differences were observed between the control (0 kg ha<sup>-1</sup>) and the highest K rate (600 kg ha<sup>-1</sup>) (Table 1). This result appeared to be related to the increase of plant vigor as a consequence of K application. Similarly to weed cover, root dry weight, root diameter, individual plant yield and stand density were not affected by P application, but they were strongly influenced by K application (Table 1). Root dry weight and individual plant yield increased with the increase of K rates. For root diameter differences were noted only between 600 kg K ha<sup>-1</sup> and the control. These results corroborated other studies that demonstrated the positive influence of K fertilization on plant vigor (Walf *et al.*, 1976; Peoples *et al.*, 1979). Additionally, a depletion of stand density was observed in consequence of K application. It appears evident that K availability increased the intraspecific competitive ability of strong healthy plants and that resulted in reducing stand density. Therefore, vigorous plants effectively compete also with weeds. As should be expected, weed cover was negatively correlated with root diameter, root dry weight and individual plant yield. Likewise, it was positively related to stand density (Figure 1).

Table 1. Effect of potassium fertilization rates on weed cover (%), stand density (plants m <sup>-2</sup> ), root
diameter (mm), root dry weight (g) and individual plant yield (g dry wt.) at the last growing season of
alfalfa harvested at flower bud stage in Legnaro, Italy from 2005 to 2007

		0 0 /	2		
$K_2O$ (kg ha <sup>-1</sup> )	Weed cover	Stand density	Root diameter	Root dry weight	Plant yield
0	74.5 a†	151 a	9.15 b	1.97 c	12.1 c
300 annually	66.2 ab	142 ab	9.61 ab	2.22 b	14.2 b
600 annually	57.5 b	131 b	10.37 a	2.43 a	16.2 a
Mean	66.1	141	9.71	2.06	19.9

<sup>†</sup>Values within a variable followed by the same letter are not significantly different at 0.05 level of probability (Fisher's protected LSD).



Figure 1. Relationship between stand density (A), root diameter (B), root dry weight (C), individual plant yield (D) and weed cover in alfalfa harvested at flower bud stage; (\*\* – significant at P < 0.01; \*\*\* – significant at P < 0.001)

#### Conclusions

Under the conditions of this study, phosphorus fertilization had no influence on weed control. Conversely, potassium fertilization had a positive effect, and especially at high rates. The lack of efficiency on weed control of phosphorus was definitely influenced by the high initial level of this nutrient in the soil. The decreased weed cover in consequence of potassium application was due to the increased vigor of the individual alfalfa plants. Potassium fertilization helps to achieve an optimum stand in which there are fewer strong plants and these compete well with weeds.

#### References

Hall M.H., Jennings J.A. and Shewmaker G.E. (2004) Alfalfa establishment guide. Online. *Forage and Grazinglands* doi:10.1094/FG-2004-0723-01-MG.

Macolino S., Lauriault L.M., Scotton M. and Ziliotto U. Response of alfalfa yield and quality to various fertilization rates in a soil lacking phosphorus or potassium deficiency. *Agronomy Journal* (submitted).

Parrini P. and Bonari E. (2002) Erba medica. In: Baldoni R. and Giardini L. Coltivazioni erbacee, Foraggere e tappeti erbosi. Patron Editore, Bologna, pp. 83–118.

Peoples T.R. and Koch D.W. (1979) Role of potassium in carbon dioxide assimilation in Medicago sativa L. *Plant Physiology* 63, 878–881.

Wolf D.D., Kimbrough E.L. and Blaser R.E. (1976) Photosynthetic efficiency of alfalfa with increasing potassium nutrition. *Crop Science* 16, 292–294.

# More efficient use of grassland under climate warming

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## Abstract

Global warming causes changes in structural and functional organization of grasslands, which, in turn, changes strategic methods for improvement of their productivity. Thus, during dry years the productivity of most grass mixtures on lowland meadows and floodplain meadows has decreased by 1–36%, but for this type of habitat the ecological and biological cenosis structure remained stable. On watershed areas productivity decreased along with the change of ecological and biological structure (cenosis xerophytization increased). The article reflects the current state of Ukrainian grasslands provision by cultivars and varieties of perennial grasses. It presents proposals for land improvement under current conditions of climate aridization according to their regional, topographical and ecological features.

Keywords: phytocenosis structure, global warming, productivity of grasslands

## Introduction

Global warming influences structural and functional features of grasslands and changes the strategic methods of grassland farming (Bohovin, 2008). Perennial grasses are mostly tolerant of increased temperature. However, they are very sensitive to lack of moisture, especially grassland species of marshland ecosystems (transpiration coefficient exceeds 600 mm). Under moisture deficits the grassland productivity decreases and perennial grasses vanish from the sward (Makarenko *et. al.*, 2005; Petrichenko *et. al.*, 2006; Kurhak, 2010; Soussana *et. al.*, 2007; Ruget *et. al.*, 2008). The reaction of different grasslands to climate aridization is variable (Bohovin, 2008). Therefore, it is necessary to take this into account when planning scientific and research activity concerning creation of the fodder resource base and during development of actions that aim to increase the productivity and effective usage of grasslands.

## Materials and methods

Research was carried out in woodland and forest-steppe zones of Ukraine on different types of fodder-producing areas. Weather conditions were evaluated with the help of humidity coefficient (HC) for flat area conditions. It was calculated as the ratio of the sum of average monthly, seasonal and annual precipitations (P, mm) to evaporation (Eo, mm) for the same

period (HC =  $\frac{P}{Eo}$ ). We calculated evaporation by formula of M.M. Ivanov: Eo = 0.0018

 $(25 + t^{\circ})^2 \times (100 - a)$ , where  $t^{\circ}$  – is the sum of monthly air temperatures and *a* is monthly average relative air humidity (Bohovin, 2008). The analysis of ecological and biological structure of phytocenosis and separate varieties was conducted using physiognomic and floristic-individualistic methods (Bohovin, 2008).

### **Results and discussion**

On lowland grasslands of woodland and on other zones of the country with hydromorphic soils, plants are supplied with water through precipitation and also from underground waters. However, the productivity of perennial grasses decreases in dry years and increases in wet years. Results of research during the dry year of 1975 (with annual humidity coefficient 0.43) showed productivity of most grass mixtures decreased by 1–36%. Greatest decrease in productivity was in mixtures which contained varieties with xerophilous inclination: *Dactylis glomerata* L., *Festuca orientalis* Hack., *Phalaroides arundinacea* L. etc. Within sufficient humidity in 1977 (with HF 1.34) productivity increased by 32–48%. Ukraine has about 3 million hectares of lowland and floodplain meadows. Within phytocenosis, mesophytes and hydromesophytes comprise about 65–70% (Table 1). The quantity of xeromesophytes and mesoxerophytes does not exceed 65–70%. Under conditions of optimal care their productivity remains stable during a long period of time. Thus, we can state that with climate aridization the quantity of xerophytes and mesoxerophytes in plants will be constantly rising. Therefore, the role of drought-tolerant varieties will increase.

On watershed habitats, where the supply of water is provided only by atmospheric precipitation, the deficit of humidity is higher. That is why the productive longevity of most perennial grasses, as recommended by State register of varieties of Ukraine (2010), is not high. Swards comprised of these species depopulate quickly (on poor soils in 2–3 years; on fertile soils in 5–6 years) and become weedy. Increased climate aridization will lead to a decrease in productivity of lowlands and floodplains. During dry years on upland meadows the dry matter yield decreases by 2–3 times, and more (e.g. on grey forest soils it decreases from 9.0–11.0 t ha<sup>-1</sup> to 4.0–5.0 t ha<sup>-1</sup>, and on dusty sandy-clay soils from 6.0–7.0 t ha<sup>-1</sup> down to 1.5–2.5 t ha<sup>-1</sup>). Later on, in the process of renewable self-regulation of zone adapted phytocenocis, productivity of these lands increases by 6.0–8.0 and 3.0–3.5 t ha<sup>-1</sup> and more. At the same time fodder quality improves.

Transformation of plant groups during 1987–2007 on a station field (Woodland), on grey forest soils was found in the direction of mesophyte group, reduction was from 52% to 3% (Table 1). Xeromesophytes were maintained in swards at a high proportion (39–48%) during all years of the research. Primarily, it was *Bromopsis inermis* Leyss., followed by *Bromopsis inermis* Leyss., *Poa angustifolia* L., *Galium verum* L., *Trifolium montanum* L. etc.). Over time the proportion of mesozerophytes and xerophytes increased from 3% to 35%, as mainly represented by *Festuca valesiaka* Gaud (Table 1).

I I	Years of phytocenosis usage							
Hygromorphs	1 <sup>st</sup>	$2^{nd}$	3 <sup>rd</sup>	7 <sup>th</sup>	14 <sup>th</sup> 8 19 40 27 6	20 <sup>th</sup>		
Xerophytes	_	5	4	2	8	45		
Mesoxerophytes	3	8	12	14	19	4		
Xeromesophytes	43	41	41	39	40	48		
Mesophytes	52	41	41	41	27	3		
Hygromesophytes	2	3	2	4	6	+		
Mesohygrophytes	_	2	_	_	_	_		

Table 1. Dynamics of ecological groups of grass varieties according to their water supply (hygromorph), years of plant group usage, % of total phytocenosis projective cover

In the Plant Varieties Register of Ukraine the mesophytes group prevails. In particular, 18 species (*Festuca pratensis* Huds., *Festuca orientalis* Hack. (*arundinacea*), *Festuca rubra* L., *Festuca ovina* L., *Phleum pratense* L., *Lolium perenne* L., *Lolium multiflorum* Lam., *Agrostis gigantea* Roth., *Elytrigia elongata* Host, *Trifolium pratense* L., *Trifolium hybridum* L.,

*Trifolium repens* L., *Lotus ucrainicus* Klok, *Medicago lupulina* L., *Lathyrus sylvestris* L., *Galega officinalis* L. and others) and by 85 varieties. This group comprises 55% of all species and 54% of all varieties that are included in the Register. The xeromesophytes group has 10 species (*Festuca orientalis* Hack., *Arrhenatherum elatius* L., *Elytrigia intermedia* L., *Bromopsis inermis* Leyss., *Dactylis glomerata* L., *Trifolium, Medicago sativa* L., *Medicago falcate* L., *Medicago varia* T. Martyn, *Melilotus album* Medik) and comprises 32% of species and 64 varieties (or 41%). Plants of this group can be used during creation of meadows from steppe lands.

Xerophytes and mesoxerophytes, which are very necessary for the steppe zone, are represented in group of perennial grasses are represented by one species of sainfoin and four and two varieties accordingly (Table 1). The group of grassland-halophilic species (as the most suitable for sowing on lowland, floodplains and heath land with salty soils) is represented only by one species – wheatgrass (one variety).

This state of issues requires expansion of species and varieties assortment of perennial grasses of Ukraine, in particular: introduction of new adapted to extreme environment species. For watershed habitat with natural moisture: *Festuca valesiaca* Gaud. i rupicola Heuff., *Agropyron pectinatum* Bieb. i desertorum Fishc., *Roegneria trachycaulon* Link, *Psathyrostachys juncea* Fichs., some species of *Astragalus, Medicago romanica* Prod., for salty meadows – *Elytrigia pseudocaesia* Pacz., *Elytrigia elongata* Host., *Puccinellia distans* Jacq., *bilykiana Klok., fomini Bilyk, bilykiana Klok* and others. Selection activity should be aimed at increase of productivity and fodder value; creation of edaphic, cenotype ecotypes, varieties with different growth rhythm. Likewise, there is a need for improvement of technologies of grassland improvement and expansion of irrigation.

#### Conclusions

The discovered peculiarities of grassland sward formations in Ukraine under conditions of climate aridization provided the basis for launching modern strategic trends in selection research, introduction and seeding of perennial grasses, as well as improvement of their technologies for usage. We should introduce and create new varieties of perennial grasses, which are characterized by high productivity and good fodder quality, resistance to droughts, cenosis resistance and good growth indicators within the season.

### References

Bohovin A.V. (2003) Ecological analysis of plant natural biogeocenosis (Physiognomic and Florist-individualistic Aspects of Ecology Analysis. *Ecology and Noospherology* 13 (1–2), 4–11.

Bohovin A.V. (2008) Effectiveness improvement of grasslands use within climate change conditions. *Volume of s. w. NRC Institute of Agriculture.* – Kyiv, pp. 33–41.

Kurhak V.H. (2010) Grassland Agrophytocenosis K.: DIA, 376 pp.

Makarenko P.S. and Petrychenko V.F. (2005) Grassland fodder production and grass seeding – Vinnytsa. Dilo, 228 pp.

Petrychenko V.F. and Kovtun K.P. (2006) Intensification trends of grassland fodder production. *Journal of Agricultural Science* 9, 24–27.

Ruget F., Abdessemed A. and Moureu J. (2008) Impact of global climate change scenarios on alfalfa production in France. *Grassland Science in Europe* 13, 745–747.

Soussana J.-F. and Luscher A. (2007) Temperate grasslands and global atmospheric change: a review. *Grass and Forage Science* 62, 127–134.

State registry of plants varieties for distribution in Ukraine in 2010 (2010). - K.: Alepha. 246 pp.

## Predicting N-fixation in a grass-clover sward

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## Abstract

The objective of this experiment was to develop a simple model to predict biological N fixation (BNF) in a white clover-perennial ryegrass sward in Ireland. Meta-analysis of international publications showed a correlation between annual BNF and the clover herbage yield: y = 33.4x + 7.1;  $R^2 = 0.92$ ; P < 0.001; where y = BNF (kg N ha<sup>-1</sup>) and x = annual white clover dry matter herbage yield (Mg DM ha<sup>-1</sup>). The ability of this equation to predict BNF was tested on a grass-clover sward in Ireland between 2008 and 2009. The slope of the regression was accurately predicted (root mean prediction error = 19.3 kg N ha<sup>-1</sup>) but the y-intercept was under-predicted (P < 0.01) and required a correction factor of + 37.0 kg N ha<sup>-1</sup>. This was due to apparent recovery of fixed-N by grass in spring which was generally higher than in the available international literature, possibly due to climatic factors. The results show that fixed-N in white clover herbage yield is predictable across studies but accurate correction factors are needed to account for fixed-N released by senescing clover material in winter and recovered by co-habiting grass in spring in various climates.

Keywords: white clover, perennial ryegrass, nitrogen fixation

## Introduction

White clover (*Trifolium repens*) facilitates biological N fixation (BNF) and is grown in grassland to reduce fertiliser costs or enable organic production. BNF is most commonly measured using either  $N_{15}$  isotopic dilution (ID) or the total-N difference method (TD). ID is more expensive and assumes no transfer of fixed-N to co-habiting grass. TD is less expensive and includes uptake of fixed-N by cohabiting grass but can be more variable (Haystead, 1993). However, BNF in grass-clover mixtures is usually associated with the amount of white clover herbage produced and therefore clover herbage yield can enable prediction of BNF (Høgh-Jensen *et al.*, 2004). The objective of this experiment was to derive a simple model from the published international literature and test the ability to predict BNF in a grass-clover sward in Ireland.

## Materials and methods

A meta-analysis of 21 international peer-reviewed publications, that specifically listed both annual BNF (kg N ha<sup>-1</sup>) and white clover herbage dry matter yield (Mg DM ha<sup>-1</sup>) in white clover-perennial ryegrass swards, was conducted, giving a total of 92 data points (contact author for full list). These were split into two categories according to whether BNF was measured using isotopic dilution (ID; 63 data points) or total N difference (TD; 29 data points). Relationships between annual BNF and annual clover yield were then analysed using linear regression with SAS<sup>TM</sup> (SAS, 2006). This relationship was also analysed on 140 grass-clover plots (each 8 m × 2 m) between June 2008 and July 2009 on a clayloam soil at the Teagasc Solohead Research Farm, Tipperary, Ireland ( $52^{\circ}51^{\circ}N$ ,  $08^{\circ}21^{\circ}W$ , 95 m a.s.l.) using the TD method (Haystead, 1993). Mean soil temperature was 9.6°C and total rainfall was 1228 mm. Herbage was cut and harvested using a lawnmower and 100 g

subsamples were taken for N-content (LECO 528 auto analyser) and DM analyses. Sward clover content (g kg<sup>-1</sup> of herbage DM) and stolon mass (kg ha<sup>-1</sup>) were measured in July, September, December, March, April and June. The area received no fertiliser N but P and K were applied to replace that removed in herbage. All relationships were compared using ANCOVA with PROC MIXED in SAS<sup>TM</sup> (SAS, 2006). Herbage production from grass-clover and grass-only plots was analysed with ANOVA in PROC MIXED.

#### **Results and discussion**

The meta-analysis revealed significant positive correlations between BNF and white clover herbage yield in the published literature. For the ID method this relationship was: y = 34.2x + 3.7;  $R^2 = 0.95$ ; P < 0.001; where y = BNF (kg N ha<sup>-1</sup>) and x = clover herbage yield (Mg DM ha<sup>-1</sup>). For the TD method this relationship was: y = 31.6x + 17.0;  $R^2 = 0.86$ ; P < 0.001. ANCOVA revealed no significant difference between the two methodologies (P > 0.05) resulting in a total regression of y = 33.4x + 7.1;  $R^2 = 0.92$ ; P < 0.001 (Figure 1a). When BNF was measured in the current experiment using the TD method, a positive correlation was again found with white clover herbage yield (y = 33.9x + 44.1;  $R^2 = 0.75$ ; P < 0.001; Figure 1b).



Figure 1. Relationships between annual white clover herbage yield and biological N fixation (BNF) for (a) the published international literature and (b) the current study. BNF measured using the total N difference method (TD; and solid trendline) or isotopic dilution (ID; and dashed trendline). No difference in slopes (P > 0.05), higher y-intercept in (b) (P < 0.001)

When this regression was compared with the previous results from the meta-analysis using ANCOVA, no significant difference in slope was found. However, the current experiment did have a higher y-intercept (P < 0.01) resulting in consistent under-prediction by the literature derived equation. Therefore a correction factor  $(+37.0 \text{ kg N ha}^{-1})$  was required, resulting in a root mean prediction error of 19.3 kg N ha-1. The need for a correction factor appeared to be due to uptake of fixed-N by the cohabiting grass in early spring as evidenced by the higher spring grass yield when grown with clover and the large net loss of clover stolon mass between autumn and spring (P < 0.001; Table 1). The proportion of fixed-N accumulated in clover stubble, stolon, roots and soil can account for approximately 70% of BNF in white clover (Jorgensen and Ledgard, 1997) and recovery by grass of 6 to 80 kg ha<sup>-1</sup> of this below-ground fixed-N has previously been recorded in Ireland (Laidlaw et al., 1996). However, the majority of international publications used the ID method and did not measure any recovery of fixed-N by grass. Of the available published data using TD, the majority (28 out of 29 data points) were from N-fertilised swards in Northern Europe. However, in Ireland a mild maritime climate with a longer grass growing season (Smit et al., 2008) may enable greater recovery of fixed-N by the cohabiting grass.

•	•							
	Au	tumn/Winter	ſ	Spring/Summer				
	(July –	(July – December 2008)			(January – July 2009)			
Measurement	Grass clover	Grass only	SEM	Grass clover	Grass only	SEM		
Clover stolon mass	1.42	_	_	0.31	_	_		
Clover herbage yield	2.71	_	-	0.76	_	_		
Grass herbage yield	3.13	3.48	0.105*	4.40	3.87	0.069***		
Total herbage yield	5.84	3.48	0.119***	5.17	3.87	0.061***		

Table 1. Herbage yields and stolon masses (all in Mg DM ha<sup>-1</sup>) measured at Solohead Research Farm between July 2008 and July 2009

\*P < 0.05, \*\*\*P < 0.001.

### Conclusions

These results show that BNF in a grass-white clover sward can be predicted from annual clover yield, with 31-34 kg N ha<sup>-1</sup> fixed per tonne of clover herbage dry matter. However, further work is needed to predict the amount of fixed-N that is lost by senescing clover over winter and recovered by the cohabiting grass in the following spring under various climatic conditions.

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### References

Haystead A. (1993) Nitrogen fixation and transfer. In: A. Davies, R.D. Baker, S.A. Grant and A.S. Laidlaw. *Sward Measurement Handbook, 2nd edn.* British Grassland Society, Reading, pp. 121–139.

Høgh-Jensen H., Loges R., Jørgensen F.V., Vinther F.P. and Jensen E.S. (2004) An empirical model for quantification of symbiotic nitrogen fixation in grass-clover mixtures. *Agricultural Systems* 82, 181–194. Jorgensen F.V. and Ledgard S.F. (1997) Contribution from stolons and roots to estimates of the total amount of N, fixed by white clover (*Trifolium repens* L.). *Annals of Botany* 80(5), 641–648.

Laidīaw A., Christie P. and Lee H. (1996) Effect of white clover cultivar on apparent transfer of nitrogen from clover to grass and estimation of relative turnover rates of nitrogen in roots. *Plant and Soil* 179(2), 243–253. SAS (2006) Version 9.1 SAS Institute. Cary, NC, USA.

Smit H.J., Metzger M.J. and Ewert F. (2008) Spatial distribution of grassland productivity and land use in Europe. *Agricultural Systems* 98(3), 208–219.

# The plasticity of functional traits drives the response of grass species to environmental changes

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## Abstract

Plant functional traits (PFTs) are useful tools to explain species' responses to environmental changes, because their values reflect the strategy whereby adaptation to variations in land use is achieved, resulting in changes in a species' performance. However, the role of PFT plasticity on plant response to environment has barely been explored. This study investigated the relationship between functional shoot traits and their plasticity due to changes in field management. Monocultures of 13 native perennial C<sub>3</sub> grasses were grown in a fully-factorial block design combining species, cutting frequency (i.e. 3 (3C) and 6 (6C) cuts y<sup>-1</sup>) and N supply (i.e. 120 (120N) and 360 (360N) kg ha<sup>-1</sup> y<sup>-1</sup>). In this study, the 3C 360N-treatment provides the optimal growth conditions and consequently allows the characterization of species strategy. Regression analyses were performed between trait values in the 3C 360N-treatments and their respective plasticity to a decrease in N supply, an increase in cutting frequency, or both. Significant regressions were found between trait values in 3C 360N and their plasticity in response to changes in management. We conclude that the ability of species to alter their morphology and/or physiology in response to varying cutting regimes or fertilisation level is both trait – (i.e. strategy indicators) and environment-dependent.

Keywords: C3 grasses, cutting, nitrogen, species strategy, trait plasticity

## Introduction

Species have been extensively described by their biological characteristics through the measurement of plant functional traits (PFTs, morpho-physio-phenological traits which influence individual performance, Violle et al., 2007). According to the similarities in the association between PFTs (i.e. trait syndrome) of their individuals, species can be grouped according to distinct functional strategies (e.g. exploitative vs. conservative species towards nutrient availability, Westoby and Wright, 2006). Moreover, evidence about the robustness of PFTs' associations (e.g. Pontes et al., 2010) lead to species classifications along environmental gradients based on unique trait values for each species. However, Albert et al. (2010) suggest that intraspecific functional variability can have significant effects on ecosystem functioning, suggesting that the strategy of a species could be further characterized by traits' responses to environmental gradients. Therefore, in addition to their contrasting trait values, species' strategy could be further characterized by traits' responses to environmental gradients. The aim of this paper is to investigate the relationship between species' functional strategies (trait values) and their plasticity in response to changes in field management. We hypothesized that for the same environmental change, species which coexist, thanks to the development of contrasting (complementary) strategies (e.g. for resource-use and response to disturbance), may present different responses based on their plasticity. Here, the term plasticity describes the variations in the magnitude of the plant trait with changes in management factors (N supply and cutting frequency).
## Materials and methods

The study was based on temperate C<sub>3</sub> grasses that co-occur in upland semi-natural mesic grasslands, representative of a wide diversity of practices (cutting or grazing, with or without fertilizer, early or late use of biomass). Thirteen species of differing stature and N acquisition/conservation trade-off were selected (see Figure 1). The species were sown in pure stands in 2001 (4.2 m<sup>2</sup> plots), and were grown in a fully-factorial block design combining species, two cutting frequencies (three or six cuts per year, 3C and 6C, respectively) and two rates of mineral N fertilizer (120 or 360 kg N ha<sup>-1</sup> y<sup>-1</sup>, 120N and 360N, respectively), with three replicates. During two years (2003–2004), 14 vegetative shoot PFTs, reflecting the morphology, phenology, physiology, and chemical composition of grass species, were measured (June and September) using standardized protocols (Cornelissen et al., 2003): leaf dry matter content (LDMC); specific leaf area (SLA); leaf length (LL); leaf lifespan (LLS); leaf N content per unit fresh matter (LNCF); number of mature leaves (NM); sheath length (SL); vegetative plant height elongated (VE); mature plant height elongated (ME); plant cellular content (CC); plant cellulose and lignin content (ADF); earliness of growth (EG); beginning of flowering period (BF); tiller density (TD). Simple regression analyses (n = 39, i.e. 13 species  $\times$  3 blocks), with Statgraphics Plus (Manugistics, USA), were performed between trait values in 3C 360N treatments (considered as offering optimal growth conditions and thus able to express the species' strategy potential) and their respective plasticity (dependent variable) to changes in i) N supply, plasticity equals ratio of trait values as [(3C 120N)-(3C 360N)]/(3C 360N); ii) cutting frequency, [(6C 360N) – (3C 360N)]/(3C 360N); or iii) in both, [(6C 120N) - (3C 360N)]/(3C 360N).

## **Results and discussion**

Significant regressions (P < 0.05) were observed between PFT values in the 3C 360N treatment (i.e. species strategy indicators) and their plasticity in response to a decrease in N supply, an increase in cutting frequency or both. In response to a decrease in N supply, *SL* ( $R^2 = 0.25$ ), *VE* ( $R^2 = 0.16$ ), *LLS* ( $R^2 = 0.32$ ) and *EG* ( $R^2 = 0.33$ ) mean trait values in the 3C 360N treatment were negatively correlated to their plasticity. The same negative correlation was shown for *CC* ( $R^2 = 0.21$ ), *ADF* ( $R^2 = 0.24$ ), *SL* ( $R^2 = 0.25$ ), *VE* ( $R^2 = 0.42$ ), *NM* ( $R^2 = 0.33$ ), *LL* ( $R^2 = 0.31$ ), *EG* ( $R^2 = 0.40$ ) and *TD* ( $R^2 = 0.14$ ) traits in response to an increase in cutting frequency. Considering both N supply decrease and cutting frequency increase, trait plasticity was negatively correlated with average values of *SL* ( $R^2 = 0.34$ ), *VE* ( $R^2 = 0.54$ ), *NM* ( $R^2 = 0.29$ ), *LL* ( $R^2 = 0.38$ ), *EG* ( $R^2 = 0.34$ ) and *TD* ( $R^2 = 0.12$ ) and positively correlated to *LNCF* ( $R^2 = 0.12$ ). Therefore, the species' responses to a new environment involve traits that vary in their strategies. In other words, the ability of species to alter their morphology and/or physiology in response to varying cutting regimes or fertilisation level is both trait- and environment-dependent.

Furthermore, the significant regressions observed in this study reveal different behaviour: (i) contrasting strategies have opposite responses to a change of field management (Figure 1a); or, (ii) a change in field management has a greater impact on a specific strategy (Figure 1b). Positive values in Figure 1 indicate an increase in trait values with a decrease in N availability. Plasticity values near to zero indicate no plasticity. Negative values show a decrease in trait values with a decrease in N availability. For instance, with a decrease in N supply, later species (low *EG* value in 3C 360N treatment, i.e. conservative species, Pontes *et al.*, 2010) become earlier, but the opposite tends to be observed for species with early growth (Figure 1a). Further, taller species (> SL), which have been associated with resistance mechanisms to cutting or grazing (e.g. Pontes *et al.*, 2010), showed a stronger plasticity



Figure 1. Relationship between trait values and their respective plasticity in response to changes in N supply (n = 39). a) EG, earliness of growth; b) SL, sheath length. Alopecurus pratensis (Ap), Anthoxanthum odoratum (Ao), Arrhenatherum elatius (Ae), Dactylis glomerata (Dg), Elytrigia repens (Er), Festuca arundinacea (Fa), Festuca rubra (Fr), Holcus lanatus (Hl), Lolium perenne (Lp), Phleum pratense (Ph), Poa pratensis (Pp), Poa trivialis (Pt) and Trisetum flavescens (Tf)

(with a negative response *i.e.* decrease in trait value) with a decrease in N supply (Figure 1b) than smaller ones (< SL). Therefore, we confirm our initial hypothesis, i.e. different responses may occur to the same environmental change according to species' strategies. Among traits, we noted that no significant regressions were found with *ME*, *BF*, *SLA* and *LDMC*. Hence, in relation to these four traits, similar responses were observed to the same environmental change for the 13 species with contrasting strategies. For instance, a decrease in N availability resulted in a decrease in *SLA* regardless of species' strategies (data not shown). We may assume that these traits are only environment-dependent, but this needs

to be confirmed in more contrasting ecological and biogeographical contexts.

#### Conclusions

With 13 co-occurring species, we showed that species plasticity is dependent on species strategy. Consequently, both trait values and trait plasticity can be informative about variations in ecosystem processes (e.g. above-ground net primary productivity). Unravelling the roles of these different components of phenotypic diversity will provide new insights into the prediction of ecosystem functioning.

#### References

Albert C.H., Thuiller W., Yoccoz N.G., Douzet R., Aubert S. and Lavorel S. (2010) A multi-trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits. *Func-tional Ecology* 24, 1192–1201.

Cornelissen J.H.C., Lavorel S., Garnier E. *et al.* (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* 51, 335–380.

Pontes L. da S., Louault F., Carrère P., Maire V., Andueza D. and Soussana J.F. (2010) The role of plant traits and their plasticity in the response of pasture grasses to nutrients and cutting frequency. *Annals of Botany* 105, 957–965.

Violle C., Navas M.L., Vile D., Vile D., Kazakou E., Fortunel C., Hummel I. and Garnier E. (2007) Let the concept of trait be functional. *Oikos* 116, 882–892.

Westoby M. and Wright I.J. (2006) Land-plant ecology on the basis of functional traits. *Trends in Ecology and Evolution* 21, 261–268.

# Behaviour of perennial grasses and alfalfa mixtures in North-Eastern Romania

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## Abstract

In Romania there are different ecological conditions, so it is necessary to develop technologies to establish temporary grassland that meet these different conditions. This paper presents results on the response of mixtures of perennial grasses and legumes, fertilized with different fertilizers, in terms of dry matter production, development of vegetation, fodder quality and nutritional status of plants. Due to its biological and physiological peculiarities, alfalfa produced high yields regardless of the weather conditions; for which reason it was used in all studied mixtures, which were afterwards fertilised with mineral and organic fertilizers and with vinassa. Based on these experimental findings, the use of mixtures in which alfalfa has a proportion of almost 50–70% in combination with chemical fertilizers is recommended.

Keywords: mixtures, grasses, legumes, fertilizer, fodder quality, nutritional status

## Introduction

The choice of species for the preparation of mixtures depends upon several criteria: adaptation to external environmental conditions, specific biological characteristics, the usage and duration of use of grassland (Cruz and Lemaire, 1986; Goliński and Golińska, 2008). Alfalfa is widely used in feed mixtures because its yields are high even in less favourable climatic conditions (Charrier *et al.*, 1993; Mosimann *et al.*, 1995). Water and heat stress lead to a decrease in production and a destabilization of the balance between the main groups of plants. This paper presents the behaviour of mixtures of perennial grasses and alfalfa, fertilized with different fertilizers, in terms of dry matter production, the development of vegetation, fodder quality and nutritional status of plants.

## Materials and methods

An experiment set up on the Ezareni farm in the spring of 2006 provided the scientific data that this paper aims to analyse and interpret. The experiment was located on terrain with a slope angle 10°, at an altitude of 107 m. The weather conditions during the experiment were characterized by an average annual temperature of 9.5°C and annual rainfall of 552 mm. The experiment was bifactorial, arranged in subdivided parcels, with four replicates and a plot size of 3 m  $\times$  5 m. The following doses of fertilizers were applied:  $a_1$  – control variant – unfertilized;  $a_2 - N_{100}P_{50}$  kg ha<sup>-1</sup>;  $a_3 - 5$  Mg ha<sup>-1</sup> vinassa +  $P_{50}$ ;  $a_4 - 30$  Mg ha<sup>-1</sup> manure applied annually. At a rate of 1000 kg manure, the chemical composition was of 5 kg N, 3 kg P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O 7 kg. Manure, phosphorus-based fertilizer, and vinassa were administered in the autumn, whereas nitrogen-based fertilizers were used twice, in the spring, before the vegetation phase started, and after the first mowing. Vinassa is a by-product obtained after the evaporation of wastewater from factories producing bakers' yeast. It has a complex chemical composition, being rich in total nitrogen (3.0%), very rich in potassium (6%) but with low phosphorus content (0.4%). The composition of perennial grass-legume mixtures, as well as the share of these grouped species, is presented in Table 1. The sampling performed depended on the type of mixture. Thus, the b<sub>1</sub>, b<sub>2</sub> and b<sub>3</sub> mixtures were harvested during the ear-emergence phase, whereas the  $b_4$  and  $b_5$  mixtures were harvested during the flowering stage of the alfalfa. Depending on the weather conditions, the type of mixture and the type of fertilization, the number of cuts obtained was between 3 and 5 per year. The changes in the structure of the vegetal canopy were determined in the spring, during the first harvest, using the gravimetric method. In order to determine the chemical composition of the fodder the following methods of analysis were used: N by Kjeldahl method; phosphorus was determined with the UV-VIS spectrometry; potassium and calcium through dosage assisted by the atomic absorption spectrophotometer (Sara and Odagiu, 2005). The statistical interpretation of production was determined through the analysis of the ANOVA variant and the comparison of the limit differences.

Crasics		Mixture						
Species	b <sub>1</sub>	b <sub>2</sub>	b <sub>3</sub>	$b_4$				
Medicago sativa L.	30	40	50	70				
Dactylis glomerata L.	25	20	20	10				
Festuca pratensis L.	15	15	10	10				
Poa pratensis L.	15	15	10	5				
Lolium perenne L.	15	10	10	5				

Table 1. Composition of tested mixtures (% by weight)

#### **Results and discussion**

Analysing the average yields achieved during the four years it appears that fertilization increased the average yields for all the tested mixtures (Table 2).

				Fertilisation	i variant			
		a <sub>1</sub>		a <sub>2</sub>		a <sub>3</sub>		a <sub>4</sub>
Mixture		Average		Average		Average		Average
	Mg ha <sup>-1</sup>	number of	Mg ha <sup>-1</sup>	number of	Mg ha <sup>-1</sup>	number of	Mg ha <sup>-1</sup>	number of
		cuts		cuts		cuts		cuts
b,	7.9	3.0	9.7*	3.3	9.4*	3.3	9.5*	3.3
b,	8.2 ns	3.3	10.5*	3.8	9.8*	3.5	10.1*	3.5
b <sub>3</sub>	8.4*	3.5	10.9*	3.8	10.4*	3.5	10.6*	3.5
b	9.2*	4.0	12.0*	4.5	11.0*	4.3	11.1*	43
LSD 0.19	% = 1.6 M	lg ha <sup>-1</sup>						

Table 2. Dry matter yield and the average number of harvests during 2007-2010

Production averages ranged between 7.9 Mg ha<sup>-1</sup> DM and 9.2 Mg ha<sup>-1</sup> DM for  $a_1$ , 9.7 Mg ha<sup>-1</sup> DM and 12.0 Mg ha<sup>-1</sup> DM for  $a_2$ , 9.4 Mg ha<sup>-1</sup> DM and 11.0 Mg ha<sup>-1</sup> DM for  $a_3$ , and 9.5 Mg ha<sup>-1</sup> DM and 11.1 Mg ha<sup>-1</sup> DM for  $a_2$ , 9.4 Mg ha<sup>-1</sup> DM and 11.0 Mg ha<sup>-1</sup> DM for  $a_3$ , and 9.5 Mg ha<sup>-1</sup> DM and 11.1 Mg ha<sup>-1</sup> DM for  $a_4$ . The average number of cuts varied between 3.0 and 3.3 at  $b_1$ , 3.3 and 3.8 at  $b_2$ , 3.5 to 3.8 at  $b_3$ , and between 4.0 and 4.5 at  $b_4$ , respectively. At  $a_1$ , the number of mowings ranged between 3.0 and 4.0, between 3.3 and 4.5 at  $a_2$ , between 3.3 and 4.3 at  $a_3$ , and between 3.3 and 4.5 at  $a_2$ , between 3.3 and 4.3 at  $a_3$ , and between 3.3 and 4.3 at  $a_4$ . In the unfertilized variant ( $a_1$ ) in all five mixtures the proportions of grass remained close to the 2007 values, with a decrease in the percentage of alfalfa plants and the appearance of forbs (Table 3). The  $a_2$  and  $a_3$  fertilization variants show an increase in the percentage contribution of grasses, the appearance of forbs, and a decrease in the percentage of alfalfa. The quality of feed produced from temporary grassland species was influenced by the components, the proportion of their contribution in the mixture, and the type and level of fertilization (Table 4). The crude fibre content of the fodder increased, while the percentage of participation of alfalfa in the fodder mixture decreased. The Ca/P ratio was good for all the fodder mixtures, and all four types of fertilization, and it has not dropped below the minimum threshold of 1.50.

	20	Fertilisation variant												
Mixture	20	07		a <sub>1</sub>			a <sub>2</sub>			a <sub>3</sub>			$a_4$	
WIIXture	Grass-	Leg-	Grass-	Leg-	Forbs	Grass-	Leg-	Forbs	Grass-	Leg-	Forbs	Grass-	Leg-	Forbs
	es	umes	es	umes	1 0103	es	umes	10103	es	umes	10105	es	umes	10103
b,	70	30	68	24	8	73	21	6	68	24	8	53	26	17
b,	60	40	62	31	7	68	28	4	63	29	8	52	32	16
b_3	50	50	56	37	7	62	34	5	54	37	7	47	38	15
b_4	30	70	33	62	5	40	56	4	37	65	9	24	64	12

Table 3. Share of grouped species (%) in 2010

Table 4. The chemical composition of fodder (2010)

Minter	Fertilisa-	Nt	PB	CF	Р	K	CaO	C-/D
Mixture	tion variant	(g kg <sup>-1</sup> )	Ca/P					
	a,	19.8	123.8	241.7	3.2	26.9	6.5	1.91
h	a,	21.8	136.3	250.1	3.5	26.7	6.4	1.88
$\mathbf{U}_1$	a <sub>3</sub>	22.2	138.8	245.1	3.4	26.8	6.3	1.85
	a	22.5	140.1	249.0	3.5	26.6	6.3	1.80
	a	22.4	140.0	240.2	3.4	26.6	6.6	1.83
h	a,	22.6	141.3	248.1	3.6	27.3	6.5	1.81
02	a <sub>3</sub>	22.8	142.5	238.4	3.5	27.5	6.6	1.89
	a	23.1	144.4	242.0	3.6	27.9	6.6	1.83
	a	23.1	144.4	232.0	3.3	26.9	6.9	1.97
h	a,	23.5	146.9	239.1	3.6	26.9	6.8	1.89
03	a <sub>3</sub>	23.6	147.5	236.5	3.6	26.9	6.9	1.92
	a	23.8	148.8	237.2	3.4	27.4	6.9	2.03
	a	24.6	153.8	222.5	3.1	27.6	7.6	2.38
,	a	24.5	153.1	233.7	3.4	26.2	7.4	2.18
0 <sub>4</sub>	a	24.8	155.0	234.6	3.3	26.4	7.5	2.27
	a,	25.2	157.5	232.8	3.3	26.6	7.7	2.33

## Conclusions

The highest average yields achieved in the four years were obtained by fertilising all five mixtures with mineral fertilizers  $(a_2)$ . Changes in the vegetal canopy were determined by the participation of mixed species and by the nature and the administered doses of fertilizers. The quality of the fodder produced from temporary pastures has been influenced by the proportion of participation in the mix of the various plant species, the type and level of fertilisation. Based on the experimental results, it is recommended that seeds mixtures are used in which alfalfa accounts for 70% of the fodder mixture. The weather conditions influenced the number of cuts obtained for all mixtures.

## References

Charrier X., Emile J.C. and Guy P. (1993) Recherche de génotypes de luzerne adaptés au pâturage. *Four-rages* 135, 507–510.

Cruz P. and Lemaire G. (1986) Analyse des relations de compétition dans une association de luzerne (*Medicago sativa* L.) et de dactyle (*Dactylis glomerata* L.). II. Effets sur la nutrition azotée des deux espèces. *Agronomie* 6(8), 735–742.

Goliński P. and Golińska B. (2008) Productivity effects of grass-legume mixtures on two soil types. *Grass-land Science in Europe* 13, 194–196.

Mosimann E., Chalet C. and Lehmann J. (1995) Mélanges lucerne-graminées: composition et fréquence d'utilisation. *Revue suisse d'agriculture* 27(3), 141–147.

Sara A. and Odagiu A. (2005) Controlul calitatii nutreturilor, Editura AcademicPres Cluj Napoca, 82-90.

# Establishment and evaluation of the productivity of ryegrass-clover swards for grazing on Derno-Podzolic sandy loam soils in the Republic of Belarus

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## Abstract

Comprehensive research has been performed on perennial ryegrass-white clover based grass mixtures for grazing, focusing on species composition and cultivars in central part of Belarus. It has been found that two-species and multi-component mixtures with Belarusian cultivars of white clover and perennial ryegrass are able to produce 4.91–5.34 t ha<sup>-1</sup> of dry matter on loamy sand. Additional associated grass species, e.g. red fescue, meadow fescue or common timothy, stabilized the productivity for a longer period. The most stable yields were obtained during the grazing period by the clover-grass mixtures consisting of perennial ryegrass cv. Pashavy and Duet, and the Festulolium cv. Punia.

Keywords: grass mixtures, cultivar assortment, perennial ryegrass, white clover, productivity

## Introduction

The lack of high quality forages and excessively high production costs per unit of forage are the main causes of low productivity and insufficient quality of livestock products (Vasko, 2005). An optimum constituent combination of legume-grass mixtures, providing productive sward longevity with a steady supply of green forage during the vegetation period can only be reached by establishment of perennial legumes based on suitable breeding and selection methods, and of course with an optimal combination of legumes in grass mixtures (Ioffe and Vasko, 2003).

## Materials and methods

Field experiments were conducted on a sod-podzol coherent sandy loam of 50–70 cm depth by coherent sand in the central zone of Belarus. The content of humus was 2.01–2.15%, pH<sub>KCl</sub> was 5.9 available P<sub>2</sub>O<sub>5</sub> was 199–232 and K<sub>2</sub>O was 201–304 mg kg<sup>-1</sup>. The plots were randomly established in four replicates with harvested area of 60 (4×15 m) m<sup>2</sup> in each plot. Sowing was carried out in the first decade of May. The mixtures were seeded after a cover crop of oats (seeding rate of 80 kg ha<sup>-1</sup>) with a seeding rate of 10.0–15.0 million viable seeds per hectare. White clover was sown across the rows of cereal grasses after soil consolidation (6.0 million viable seeds per hectare seeding rate). A cover crop of green forage was harvested at the ear- formation stage. The mixtures were cut 4 times per year. The intervals between cutting the first growth and the aftermath comprised 25 days, between the aftermath and the third cut 25–30 days, and between the third and the fourth 35–40 days, depending on weather conditions.

## **Results and discussion**

In the experiment it was found that the two-and multi-component grass mixtures gave 4.98 t  $ha^{-1}$  of as DM per ha, and grass mixtures with additional cereal components increased the yields to 4.91–5.34 t  $ha^{-1}$  (Table 1). The greatest DM productivity among multi-component grass stands was generated by a five-component grass mixture (Table 1).

	• •	e 1	-		
		Dry matte	r yield, t ha <sup>-1</sup>		
Mixtures	1 <sup>st</sup> year of utili-	2 <sup>nd</sup> year of	3rd year of utili-	Maan	
	zation	utilization	zation	Mean	
Perennial ryegrass + white clover	6.42	4.91	3.60	4.98	
Perennial ryegrass + white clo-	6.13	5 46	3 00	5 16	
ver + red fescue	0.15	5.40	5.90	5.10	
Perennial ryegrass + white clo-	6.15	5 32	4 01	5 16	
ver + meadow fescue	0.15	5.52	4.01	5.10	
Perennial ryegrass + white clo-	5 84	4 87	4 02	4 91	
ver + timothy	5.04	4.07	7.02	7.71	
Perennial ryegrass + white					
clover + red fescue+ meadow	6.32	5.65	4.06	5.34	
fescue+ timothy					
LSD <sub>0.05</sub>	0.46	0.44	0.28	_	

Table 1. Pasture grass mixtures productivity depending on species composition

The yields of simple mixtures with only 2 components (Lolium perenne and Trifolium repens) were not stable over the years, and the yield decreased by the third year compared with that of the mixtures with more components. Unfavourable weather conditions for perennial ryegrass and white clover in the winter of 2004–2005 and in the springtime of 2005 thinned out grass stand and did not allow high productive grass swards in the third year. Two-component mixtures with ryegrass and clover ranked below the multi-component grass stands in terms of DM productivity; this was associated with sward thinning of the ryegrass and white clover due to winter conditions. Our research on botanical structure showed that perennial ryegrass in the ryegrass-clover grass stand combined well with red fescue (Festuca rubra; mid ripening) and common timothy (Phleum pratense; late-ripening). The proportions of perennial ryegrass in the grass mixtures varied between 32.4 and 35.6%. Meadow fescue (Festuca pratense; mid-ripening) shows strong coenotic activity in ryegrass-clover grass stands and depresses the perennial ryegrass to a greater extent than meadow fescue and common timothy, reducing the proportion of ryegrass to 25.3% in a grass mixture. White clover content in grass stands for 3 years of use averaged between 37.7 and 42.4% depending on the grass mixture. White clover coenotic activity increases if there is sufficient plant-moisture supply and warm weather. In our research, studying the crop formation dynamics by various perennial ryegrass cultivars has shown that the perennial ryegrass late-ripening cv. Pashavy grows slowly in the first grazing cycle and has the advantage on productivity in the second cycle. Festulolium possesses high regrowth rates in the first cycle. Mixing of perennial ryegrasses Pashavy, Duet, and Festulolium Punia cultivars with asynchronous growth rhythms and biomass accumulation in one multi-component ryegrass-clover grass mixture provides more uniform supply of green forage in the grazing period.

## Conclusions

On sod-podzol coherent sandy loam the addition of red fescue, meadow fescue or common timothy as associated grasses in a ryegrass-white clover pasture mixture resulted in more stable productivity over time. Mixing of perennial ryegrass Pashavy, Duet, or Festulolium Punia cultivars with asynchronous growth rhythms and biomass accumulation in one multicomponent ryegrass-clover grass mixture provides a more uniform supply of green forage during the grazing period.

## References

Ioffe V.B. and Vasko P.P. (2003) Cultivated pastures: Practical recomendation, UP Print. 'Victory', Molodechno, Belarus, 120 pp.

Vasko P.P. (2005) The system of Belarusian cultivars of white and red clover, as a stabilizing factor of herbaceous forage production. *Agriculture and Plant Protection* 6, 5–7.

# Nutritive value and early yield formation of legume-grass swards in a crop rotation

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## Abstract

The aim of this experiment was to ascertain the early herbage production of swards grown under different competition conditions. Legume-grass mixtures were sown with and without a cover crop of barley or peas for whole-crop and as barley for grain. *Trifolium pratense* L. and *Medicago sativa* L. were sown in mixtures with *Lolium perenne* L. The total yields over two years differed due to the composition of leys and weather conditions. In drier years, lucerne-ryegrass swards were in all cases significantly more productive than red clover-ryegrass swards. In wetter years red clover-ryegrass swards were significantly more productive than lucerne swards, except for lucerne-ryegrass swards grown without a competitive cover crop. The concentration of crude protein (CP) in herbage met the needs of dairy cows. During the two years there was a higher CP content in the swards with a pea cover crop. The highest digestible protein amount was observed in clover-ryegrass swards with a barley cover crop, and lucerne-ryegrass without a cover crop.

Keywords: clover, lucerne, botanical composition, yield, protein

## Introduction

Legumes are one of the main groups of plants for improving sustainability, stability of agroecosystems, and they provide the cheapest resource of nitrogen (Wilkins and Vidrih, 2000). The ability of legumes to fix  $N_2$  can be exploited to increase yields, growing rates and quality of sward mixtures. However, experimental evidence shows that, as a forage crop grows, its yield increases but the nutritive value tends to decrease (Kleen *et. al.*, 2011). Botanical composition of grassland is very important for forage quality. Forage digestibility is one of the main characteristics that determines nutrient supply and consequently animal performance and there is a need to determine the chemical components most closely relate to these (Cherney and Mertens, 1998). Mixtures of ryegrass and clover could increase the forage quality, and milk production can be improved by 15–25% compared with pure perennial ryegrass swards (Phillips *et al.*, 2000). The aim of the current study was to determine yield formation rate and quality of legume-grass leys, grown under different competition conditions, and to ascertain their productity in swards over two years.

## Materials and methods

A field experiment was conducted on a loamy *Endocalcari-Epihypogleyic Cambisol* in Dotnuva (55° 24' N) with trials over two years (in 2003–2004, and in 2004–2005). Soil pH varied between 6.5 and 7.0; soil organic matter content was 2.5–4.0 per cent; available P was 50–80 mg kg<sup>-1</sup> and K 100–150 mg kg<sup>-1</sup>. Legume-grass mixtures were sown with and without a cover crop of barley (*Hordeum vulgare* L.) or peas (*Pisum sativum* L.) for grain and whole-crop respectively. Red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.) were sown in mixtures with perennial ryegrass (*Lolium perenne* L.) and compared with pure-stands of ryegrass without fertilisation. The experiments had a randomised block design with four replicates. The yield was measured at the blooming stage of legumes. The swards were cut twice in the first year and 3 times in the second year. Peas and barley as whole-crops were harvested at the wax and grain 'greasy' stage, respectively, and for one treatment – barley for grain – at the complete-ripeness stage. The data were statistically processed using analysis of variance. In 2003, the conditions for growth and re-growth were somewhat droughty, with a shortage of moisture for potential growth. In 2004, the spring and early summer were also somewhat droughty, but since there was no shortage of moisture later, the season overall was favourable for grass growth.

## **Results and discussion**

The yield structure varied with botanical composition and plant species contribution to the yield. Lucerne without a cover crop had the highest yield in perennial swards (Table 1).

-		-			-			
Swand		1 <sup>st</sup> year				2 <sup>nd</sup> year		_
cover crop	Legumes	Grasses	Forbs	Cover crops	Legumes	Grasses	Forbs	Cover crops
Rcl/Pr	50.1	30.3	19.6		50.1	43.1	6.8	
Rcl/Pr/Bgr	56.2	17.6	26.3		66.4	30.5	3.1	
Rcl/Pr/Bwc	34.7	22.0	3.4	39.9	60.8	13.6	0.8	24.8
Rcl/Pr/Pwc	32.3	30.7	14.8	22.2	47.1	30.1	2.4	20.4
Lc/Pr	78.5	10.6	10.6		51.0	47.1	1.9	
Lc/Pr/Bwc	40.3	21.2	3.7	34.8	39.7	33.9	3.0	23.4
Lc/Pr/Pwc	65.3	12.8	10.0	11.9	39.0	41.4	3.9	15.7
Pr N <sub>o</sub>	0	79.7	20.3		0	95.2	4.8	

Table 1. The impact of sward composition and sowing method on dry matter yield weighted average over two years of use, expressed as % of total annual DM yield

Rcl-red clover, Lc-lucerne, Pr-perennial ryegrass, Bgr-barley for grain, Bwc-barley for whole crop, Pwc-peas for whole crop, Pr  $N_0$ -pure perennial ryegrass stand.

Lucerne is an important fodder legume under dry conditions, because this plant has a deep tap root and can take up water from the sub-soil (Pietsch *et al.*, 2007). The treatment of lucerne with a cover crop of peas accounted for a higher share in the DM yield. The cover crop of barley for whole-crop suppressed the growth of legumes and they accounted for a much smaller share in the DM yield than without a cover crop or with a cover crop of peas. Assessment of the total yield of two years in both trials showed that there existed significant differences between the three different swards with different cover crops or without them (Figure 1).

The total yield of lucerne-ryegrass did not differ between treatments sown with or without a cover crop. This total yield was about 50% higher than that of the clover-ryegrass mixture. Lucerne ley with a cover crop of peas accumulated a slightly higher (P < 0.05) DM yield compared with a cover crop of barley. The total of two-years' DM yields of the swards was highly dependent on the sward species. CP concentration met the needs of cattle in all the cases studied. CP also depended on cover crop and legume species in the first growing year of leys (Table 2).

In the sowing year of leys, the highest amount of CP was found in the yield of lucerne with a pea cover crop. The amount of digestible protein (DP) varied: the lowest content was determined in the pure ryegrass stand. There was a significantly negative effect (P < 0.05) of cover crop, especially of barley, on the DP yield in lucerne-ryegrass. ME amount corresponds closely with DM yield but there were some variations between treatments. In the first-year trials, leys with lucerne had significantly (P < 0.05) higher ME value than leys with red clover. In the second-year trial, red clover and perennial ryegrass leys, sown with



Figure 1. Dry matter yields of swards as affected by a cover crop and sward composition, kg ha<sup>-1</sup> P < 0.05– significant differences (ANOVA, LSD-Test)

a barley cover crop for whole-crop, accumulated significantly (P < 0.05) larger amount of ME compared with all other investigated leys.

Table 2. Crude protein (CP) yield, amount of digestible protein (DP) and metabolisable energy (ME) in the total two years' yield of swards

Treatments:		1 <sup>st</sup> trial			2 <sup>nd</sup> trial	
sward+ cover crop	CP kg ha <sup>-1</sup>	DP kg ha <sup>-1</sup>	ME GJ ha <sup>-1</sup>	CP kg ha <sup>-1</sup>	DP kg ha <sup>-1</sup>	ME GJ ha <sup>-1</sup>
Rcl+Pr	1664	1162	105	2446	1636	161
Rcl+Pr+Bgr	1810	1317	128	1847	1293	145
Rcl+Pr+Bwc	1640	1135	95	2762	1887	177
Rcl+Pr+Pwc	1732	1234	118	2522	1733	168
Lc+Pr	3082	1973	167	2695	1765	162
Lc+Pr+Bwc	2546	1626	163	1674	1133	111
Lc+Pr+Pwc	3042	2012	174	1847	1282	128
Pr N <sub>0</sub>	577	383	50	1555	1004	101
P < 0.05	158.0	109.2	10.2	178.5	123.8	11.1

P < 0.05 – significant differences, ANOVA, LSD-Test.

## Conclusions

In drier years, lucerne-ryegrass swards were significantly more productive than red clover-ryegrass swards in all the cases studied. However, in wetter years lucerne-ryegrass swards were significantly less productive than red clover-ryegrass swards, except for lucerne-ryegrass swards that grew without a competitive cover crop. Lucerne-ryegrass swards appeared more sensitive to effects of the competitive cover crop than red clover-ryegrass swards. All swards with a cover crop of peas were distinguished by having higher CP in the first year.

## References

Cherney D.J.R. and Mertens D.R. (1998) Modelling grass utilisation for dairy cows. In: Cherney J.H. and Cherney D.J.R. (eds) *Grass for Dairy Cattle*, CAB International, Wallingford, Oxon, UK, pp. 351–371.

Kleen J., Taube F. and Gierus M. (2011) Agronomic performance and nutritive value of forage legumes in binary mixtures with perennial ryegrass under different defoliation systems. *Journal of Agricultural Science* 149, 73–84.

Phillips C.J.C., James N.L. and Nyallu H.M. (2000) The effects of forage supplements on the ingestive behaviour and production of dairy cows grazing ryegrass only or mixed ryegrass and white clover pastures. *Journal of Animal Science* 70, 555–559.

Pietsch G., Friedel J.K. and Freyer B. (2007) Lucerne management in an organic farming system under dry site conditions. *Field Crops Research* 102(2), 104–116.

Wilkins R.J. and Vidrih T. (2000) Grassland for and beyond 2000. Grassland Science in Europe 5, 9-17.

## Herbage yield and botanical composition of Italian ryegrass forage crops associated with different types of nitrogen supply

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## Abstract

Italian ryegrass monoculture and mixtures with red clover (sowing ratios 75:25%, 50:50% and 25:75%) were established in the spring of 2008 at the Institute for Animal Husbandry, Belgrade, with the aim to analyse the possibilities of managing the N supply. There were four N rates for Italian ryegrass monoculture: 0, 100, 150 and 200 kg ha<sup>-1</sup>, whilst no N fertilizer was applied to the mixture plots. The trial was carried out during 2008–2010 and the highest total DM yield was obtained with 200 kg N ha<sup>-1</sup>, similar to that of the ryegrass-clover mixtures. Ryegrass contributed less to the total yield in the mixtures, because it had lower ratios in the structure of swards than those used at sowing. An Italian ryegrass-red clover sward may have advantages over a pure Italian ryegrass sward fertilized by high rates of N, providing high yield with a more sustainable land-use system.

Keywords: botanical composition, Italian ryegrass, nitrogen fertilizer, red clover

## Introduction

Modern trends in Europe are promoting the use of grass-clover mixtures to produce more farm-grown protein and to decrease the use of mineral fertilizers (De Vliegher and Carlier, 2008). Unsown species contribute progressively increased proportions of the total yield of sown monocultures, than in the mixtures, where the invasion of unsown species is minimal. When seeded with legumes, Italian ryegrass (IR) provides early protection of the soil, suppresses weeds, and acts as a nurse crop. The N content is the main individual nutritional factor affecting the growth and development of Italian ryegrass (Griffith and Chastain, 1997). Nitrogen acquisition is one of the most important factors for plant production, and N contribution from biological N<sub>o</sub> fixation can reduce the need for industrial N fertilizers (Carlsson and Huss-Danell, 2003). When grown in mixtures with grasses, red clover (RC) obtained a high proportion of its nitrogen from N, fixation (average around 80%), regardless of management, dry matter yield and location. Wacquant et al. (1989) suggest that NO3 enrichment in the soil results from N excretion from active nodulated roots of the legume, accounting for the increase in both biomass and nitrogen content of the companion grass in grass-legume associations. Introduction of high rates of N fertilizer can also lead to accumulation of NO, in plants, and also in soil, which can have negative consequences (Nesic et al., 2008). Thus, the aim of the present study was to evaluate the influence of the Italian ryegrass management and N supply on yield and botanical composition of herbage for the design of strategies of management allowing a more sustainable land use system.

## Materials and methods

The study was carried out during 2008–2010 at the Institute for Animal Husbandry, Belgrade-Zemun. Italian ryegrass (cv. K-29t) was sown in March 2008, alone and with red

clover (cv. Una) using different proportional ratios of IR:RC (75:25%, 50:50% and 25:75%). Italian ryegrass sown alone received nil or 100, 150 and 200 kg ha<sup>-1</sup> N, whereas no N fertilizer was applied to the IR-RC mixtures. Sowing rates were 30 kg ha<sup>-1</sup> of Italian ryegrass and 15 kg ha<sup>-1</sup> of red clover. The trial was established in a randomized complete block design of plots (each  $3 \times 4$  m) with 3 replicates. The low development rhythm of IR and RC in the first year of vegetation (a dry spring) permitted the establishment of weeds, and the crops were harvested twice without any assessment of production. For the assessment of dry forage production and botanical composition, each plot was harvested twice per year (2009–2010), and a composite sample was taken at each harvest from all the treatments. Botanical composition of the plots was determined and classified as grass, legumes and others based on dry weights. Data were analysed by parametric tests (ANOVA and LSD test) at the 0.05 probability levels, using the statistical package Statistica 8. The main characteristics of the soil (depth: 0–20 cm) were the following: soil texture: clay-loam; pH 1M KCl: 5.33; total N: 0.19%; K<sub>2</sub>O: 18.4 mg kg<sup>-1</sup> and P<sub>2</sub>O<sub>5</sub>: 5.4 mg kg<sup>-1</sup>. According to the http://www.hidmet.gov.rs the area of study is characterized by a temperate climate with a mild summer dry season.

## **Results and discussion**

The dry matter (DM) yield of pure-sown IR swards in the both years was largely dependent on the fertilization (Table 1). The first production year was characterized by a higher DM yield of the control and lower N rate (100 kg ha<sup>-1</sup>) treatments, in comparison with the second year. Higher N rates improved the IR herbage yield in the second year, especially at the second cut. There were significant differences among N application rates in the second experimental year: treatments with the highest amount of N resulted in a threefold higher yield than the treatment without N. Treatments with N fertilizer resulted in considerably higher yield in comparison with the control treatment. Treatments without N had the lowest yield in both years. No significant response was detected of DM yield to the different ratio of components in the mixtures. Considering the obtained yields on the cumulative DM, a high yield was achieved for all mixtures of IR+RC, which was similar to the yield of the pure sown IR with the highest added rate of N fertilizer. IR without any N supply did not achieve a satisfactory level of herbage yield.

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Traatmont		2009			2010		2009-2010
Treatment	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	Total DM	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	Total DM	Cumulative DM
IR + 0 kg ha <sup>-1</sup> N	2230°	212°	2442°	1525 <sup>d</sup>	383°	1908 <sup>d</sup>	4350°
IR + 100 kg ha <sup>-1</sup> N	3831 <sup>ab</sup>	235°	4066 <sup>abc</sup>	2743°	366°	3109°	7175 <sup>b</sup>
IR + 150 kg ha <sup>-1</sup> N	3122 <sup>bc</sup>	479°	3601 <sup>bc</sup>	4013 <sup>b</sup>	$478^{bc}$	4491 <sup>b</sup>	8092 <sup>ab</sup>
IR + 200 kg ha <sup>-1</sup> N	4389 <sup>ab</sup>	309°	4698 <sup>ab</sup>	5380ª	898 <sup>a</sup>	6278ª	10976ª
IR + RC (75:25%)	3839 <sup>ab</sup>	1017 <sup>b</sup>	4856 <sup>ab</sup>	5043 <sup>ab</sup>	802 <sup>ab</sup>	5845ª	10701ª
IR + RC (50:50%)	4077 <sup>ab</sup>	1553ª	5630ª	5193ª	791 <sup>ab</sup>	5984ª	11614ª
IR + RC (25:75%)	4612ª	1275 <sup>ab</sup>	5887ª	5050 <sup>ab</sup>	1024 <sup>a</sup>	6074ª	11961ª
LSD <sub>0.05</sub>	1479	386	1824	1078	395	1097	2262

Table 1. DM yield of Italian ryegrass as pure stands and mixture with red clover (kg ha<sup>-1</sup>) during the 2009 and 2010 growing seasons

IR had lower ratios in the structure of swards than those used at sowing, both at the first and the second cut (Figure 1). Red clover was absolutely dominant in the second cuts of both years, and this can be explained by the dry weather during summer growth, conditions leading to poor regrowth of ryegrass and more favourable for clover. Weeds contributed 4–35% of the botanical composition of all treatments at the first cut, while at the second cut their proportions decreased to less than 10% in the majority of variants. The ratios of weeds decreased in the botanical composition of swards of all mixtures by the second cut; this result being in agreement with findings reported by De Vliegher and Carlier (2008).



Figure 1. Botanical composition of sward (%) with pure sown Italian ryegrass (IR) and mixture with red clover (RC)

#### Conclusions

It could be concluded that high forage yield could be obtained without N fertilization, using a suitable legume component in mixture with Italian ryegrass. Different sowing ratios of IR+RC mixtures did not result in significant differences in cumulative yield over two years; moreover, they had a relatively constant level of yield, which could exceed the yield of pure-sown Italian ryegrass with high N supply, but the proportions of the components were not stable during the production period, since red clover strived to be more dominant in mixtures. The use of red clover in mixtures with Italian ryegrass can be a suitable method to reduce requirements for nitrogen fertilizers.

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## References

Carlsson G. and Huss-Danell K. (2003) Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil* 253, 2, 353–372.

De Vliegher A. and Carlier L. (2008) Potential of fodder legumes under intensive farming conditions in Flanders. *Grassland Science in Europe* 13, 236–238.

Griffith S.M. and Chastain T.G. (1997) Physiology and growth of ryegrass. In: *Ecology, Production, and Management of Lolium for Forage in the USA*, F.M. Rouquette, Jr. and L.R. Nelson (eds), CSSA Special Publication Number 24, Madison WI, 15–28.

Nesic Z., Tomic Z., Ruzic-Muslic D. and Vuckovic S. (2008) Effect of seed mixture and N fertilization on nitrate content of grass-legume swards. *Grassland Science in Europe* 13, 429–431.

Wacquant J.P., Ouknider M. and Jacquard P. (1989) Evidence for a periodic excretion of nitrogen by roots of grass-legume associations. *Plant and Soil* 116, 57–68.

# White and red clover in highly productive short-lasting grassland mixtures

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## Abstract

With the aim of increasing home-grown crude protein production, a plot experiment was carried out on two dairy farms with five different seed mixtures of red clover (*Trifolium pratense*) and/or white clover (*Trifolium repens*) together with grass. The clover constituted in total 20% of the seed weight. There were three further N-application rates. The dry matter and the crude protein yield increased with an increasing proportion of red clover and a decreasing proportion of white clover in the seed mixture. This seems primarily to be an effect of a higher competitive strength of red clover. The red clover proportion in the dry matter seemed to be more dependent on the amount of seed in the mixture than white clover. With increasing N-application the dry matter yield increased, but the crude protein yield decreased. This decrease in crude protein yield was primarily due to an increasing proportion of grass with a low content of crude protein. The crude protein content of red and white clover was high and independent of N-rate during the whole growing season. The crude protein content of the grass increased with increasing N-rate in spring, but in the last part of the growing season the crude protein content decreased with increasing N-rate, which probably was due to a high N-transfer from the clover.

Keywords: seed mixture, N-rate, crude protein

## Introduction

In recent years there has been an interest in increasing home-grown protein production on dairy farms. Legumes play an important role in this. In cutting systems, red clover is well known to be a highly productive grassland species with a high competitive strength. White clover has a long tradition of being an important part of the sward, but with increasing use of cutting and decreasing use of grazing together, with an increasing use of festulolium (*xFestulolium*) as the companion grass, which strongly competes with white clover (Søegaard and Weisbjerg, 2007), the proportion of clover in the sward is under pressure. Red clover cannot spread through stolons, and therefore red clover seed number is an important parameter for the potential proportion of red clover in the herbage. In contrast, white clover has stolons and therefore the seed number has less significance. Seed mixtures with both red and white clover could probably optimize the benefits of both in the grassland production. We therefore established an experiment with the same proportion of clover seed in the mixture but with different ratios between white and red clover.

## Materials and methods

Plots with five different seed mixtures with a seed rate of 25 kg ha<sup>-1</sup> were established in autumn 2008 on two dairy farms with coarse sandy soil and sandy loam, respectively. All seed mixtures contained in total 20% of white clover (cv. Klondike) seed and/or red clover (cv. Rajah) seed in the combinations: 20White clover/0Red clover, 15W/5R, 10W/10R, 5W/15R and 0W/20R. The 80% grass seed was composed of 30% perennial ryegrass (*Lolium perenne* cv. Option) and 50% festulolium (cv. Perun). There were three rates of N-mineral fertilizer (N-rate to the single cut are shown in brackets): 0 N, 110 N (50-40-20-0) and 220 N ha<sup>-1</sup> (100-80-40-0). There were four replicates. In spring all plots were fertilized with 24 kg P and 120 kg K ha<sup>-1</sup>. The fields were irrigated at drought stress. In 2009 and 2010 four cuts were harvested with a Haldrup plot harvester and botanical composition was determined by hand separation in subsamples of 200 g weight and calculated as weighted means. Concentration of N in the single species was measured by Dumas method and crude protein was calculated as  $6.25 \times \%$ N. The plots were randomly placed in a block design.

## **Results and discussion**

The swards were well-established on both farms, which was also reflected in a low proportion of unsown species (Figure 1). Red clover competed more strongly with grass than white clover (Figure 1), and this was the case during the whole growing season (data not shown). Without N-application there was 53% white clover and 73% red clover in the dry matter for mixture 20W/0R and 0W/20R, respectively. At the other N-rates the same effects of seed mixtures were found, but with an increasing proportion of grass (Figure 1). N-rate seemed not to affect the competition between white and red clover, as the N-rate had nearly the same relative effect on the decrease of the two legumes. In the seed mixture without white clover the unsown species was primarily white clover germinated from white clover seeds in the soil.



Figure 1. Mean botanical composition (as % of herbage dry matter) of seed mixtures at different N-rates. 20W/0R = 20% white clover and 0% red clover in the seed mixture

The content of crude protein was high in both white and red clover through the whole growing season and was a little higher in white clover in the last part of the season (Table 1A). N-rate had no significant effect on the crude protein content in either white or red clover (data not shown). The content of crude protein in grass was, however, low in spring and increased highly during the season, and the N-rate had a significant effect on this increase (Table 1 B). In cut 1 the crude protein content in grass increased with N-rate. During the growing season the crude protein content increased at all N-rates, but the increase was highest without N-application and lowest at the highest application (Table 1B). This is probably a combined effect of N-application and N-transfer from clover to grass in such a way, that in the beginning of the season there was mostly a direct N-fertiliser effect; whereas later, the N-transfer from clover to grass increased the content of crude protein in the grass, and this transfer was increased with decreasing N-rate due to the increasing proportion of clover. The dry matter yield increased significantly with increasing N-application (P < 0.0001) (Figure 2A). The dry matter yield increased further significantly with increasing proportion of red clover and decreasing proportion of white clover in the seed mixture (P = 0.03). This increase was highest at 0N.

Table1. Concentration of crude protein (g kg <sup>-1</sup> DM). A: In the different species during the	season as
mean of N-rates, and B: in the grass at the different N-rates during the season	

A (species)	Cut 1	Cut 2	Cut 3	Cut 4	B (grass)	Cut 1	Cut 2	Cut 3	Cut 4
Grass	61 <sup>b</sup>	78 <sup>b</sup>	132°	159°	0 N	57 <sup>b</sup>	80 <sup>a</sup>	173ª	198ª
White clover	222ª	212ª	252ª	280 <sup>a</sup>	110 N	55 <sup>b</sup>	68 <sup>b</sup>	120 <sup>b</sup>	158 <sup>b</sup>
Red clover	227ª	207 <sup>a</sup>	221 <sup>b</sup>	253 <sup>b</sup>	220 N	70 <sup>a</sup>	86ª	102°	122°

Different letters show significant differences (P < 0.05) within species (A) and N-rates (B).

In contrast, the yield of crude protein was highest at 0 N (P = 0.02) and the protein yield was not significant different between 110 N and 220 N (P > 0.05) (Figure 2B). The protein yield increased significantly with an increasing proportion of red clover in the seed mixture (P = 0.01). The main reason for these findings must be found in the effects on the botanical composition and content of crude protein (Figure 1 and 1A). The proportion of grass decreased with an increasing proportion of red and decreasing proportion of white clover in the seed mixtures (Figure 1). Averaged across mixtures the grass proportions were 32, 61 and 78% of dry matter at 0, 110 and 220 N, respectively. At the same time the content of crude protein was 203, 159 and 142 g kg<sup>-1</sup> dry matter (data not shown). These effects gave in combination the contrasting dry matter-crude protein results.



Figure 2. Annual yield of dry matter (A) and crude protein (B) in different seed mixtures and N-rates. Mean of two farms and two years

#### Conclusions

A high proportion of red clover in the seed mixture, either red clover alone or together with a small proportion of white clover, gave both the highest yield of dry matter and crude protein. However, it was not possible to optimize both the dry matter and the protein yield, as the highest crude protein yield was found in the unfertilized crop. It seems therefore necessary to choose between maximizing either the protein yield or the dry matter yield.

#### References

Søegaard K. and Weisbjerg M.R. (2007) Herbage quality and competitiveness of grassland legumes in mixed swards. *Grassland Science in Europe* 12, 166–169.

# Lucerne varieties for continuous grazing

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## Abstract

In recent years there has been a growing interest, particularly in organic farming, for grazing lucerne (*Medigaco sativa*), partly due to problems with white clover fatigue and partly to the wish for higher biodiversity. Therefore, three grazing-tolerant varieties were examined under relatively severe grazing with heifers in two cutting/grazing managements. Two new varieties, Verbena and Camporegio, and an older variety Luzelle were established in 2009 in pure stands and in two different mixtures with perennial ryegrass (*Lolium perenne*). Camporegio had the lowest yield, the lowest competitive strength, the lowest plant density in spring, and the density was most reduced during grazing. The results could not confirm significant differences between the new and the older varieties. The results for Luzelle were generally between Verbena and Camporegio. The varieties did not differ in herbage quality either under grazing or cutting.

Keywords: lucerne, varieties, grazing

## Introduction

In Danish grazed grasslands, white clover (*Trifolium repens*) is the most important legume. It grows and competes very well under these conditions. Especially in organic grasslands a high legume proportion is essential to ensure high soil fertility. In recent years there has been a wish for incorporating other legumes, due both to problems with white clover fatigue in intensive cultivated grass-clover areas (Søegaard and Møller, 2005) and the wish for a higher biodiversity. Grazing-tolerant lucerne could be a potential species, as studies have indicated that lucerne is not as liable to clover fatigue. With the purpose to examine the suitability of new grazing-tolerant lucerne varieties in intensively used grasslands, three varieties were compared at different managements.

## Materials and methods

Lucerne plots were established in spring barley (*Hordeum vulgare*) on sandy loam in spring 2009. There were three varieties: Verbena, Camporegio and Luzelle, which were established with three seed mixtures all sown with 25 kg seed ha<sup>-1</sup>: 10 kg lucerne and 15 kg grass (10L/15G); 15 kg lucerne and 10 kg grass (15L/10G); and 25 kg lucerne (25L/0G). The grass mixture comprised 40% Sibasa, an intermediate diploid perennial ryegrass, and 60% Maurice, a late tetraploid perennial ryegrass. The growing season was split up in three periods: spring (spring–10 June), summer (10 June–10 August) and autumn (10 August–11 October). There were two managements in these periods: cut-grazing-cut (C-G-C) and grazing-cut-grazing (G-C-G). The lucerne was inoculated with Rhizobia before sowing. To ensure that K would not be growth-limiting, the plots were fertilized with 200 kg K; 100 kg in spring and 100 kg in mid June. In the grazing periods pregnant heifers grazed the plots continuously to a sward height of approximately 5 cm. The plot size was 24 m<sup>2</sup> for the grass-lucerne plots and 12 m<sup>2</sup> for the lucerne in pure stand. Cut yield was determined by a Haldrup plot harvester and botanical composition determined by hand separation of subsamples. Samples under grazing were torn-off by hand. Herbage quality of lucerne

was determined at NIR/Research Automation group, LVH Rilland Centre of Expertise. An indication of plant density was determined as the distance from ten random points to the nearest lucerne plant per plot.

## **Results and discussion**

The yield under cutting was slightly lower in Camporegio than the other varieties, especially in cuts 2 and 3 (Table 1). The yield of lucerne in pure stand was slightly lower than in mixture with grass, on average by 5% (data not shown).

Table 1. Dry matter yield (t ha<sup>-1</sup> DM) under cutting at two different managements, mean of three seed mixtures. Yield of cut 1 and 3 from management (cutting-grazing-cutting) and cut 2 from (grazing-cutting-grazing)

	Cut 1	Cut 2	Cut 3
	(C-G-C)	(G-C-G)	(C-G-C)
Verbena	4.92	5.36 <sup>b</sup>	1.83ª
Camporegio	4.76	4.99°	1.58 <sup>b</sup>
Luzelle	4.43	5.49ª	1.87ª

Different letters indicate significant differences (P < 0.05) between varieties within cut.

The herbage quality was the same for all three varieties (Table 2). There were no significant differences. There was a relatively high crude protein concentration in general, and for cell wall and lignin concentrations there were lower differences between grazing and cutting treatments than expected (Søegaard and Weisbjerg, 2007).

Table 2. Herbage quality (g  $kg^{-1}$  DM) measured only in the lucerne part of the herbage. Average over season. Crude protein (CP), water soluble carbohydrates (WSC), cell wall (NDF) and lignin (ADL)

		Gra	zing		Cutting				
	CP	WSC	NDF	ADL	CP	WSC	NDF	ADL	
Verbena	278	56	364	40	220	31	419	49	
Camporegio	261	59	365	40	228	27	425	47	
Luzelle	288	48	361	39	226	39	411	47	

Botanical composition differed greatly during the season. As expected there was a large white clover seed bank in the soil due to many years of growing white clover-grass swards. Therefore, herbage mass was also split into an unsown white clover proportion. In spring the proportion of unsown species was limited, and lucerne in pure stand constituted more than 90% of herbage dry matter (Table 3). The plots at cut 1 had not been grazed. The plots at cut 2 had been grazed in the spring period, and at cut 3 the plots had been grazed in the summer period. The proportion of lucerne decreased from cut 1 to cut 2, and decreased even more in cut 3. The proportion of white clover and other unsown species was high in autumn, which indicates that there had been room for them to develop. Thus, grazing weakened the lucerne growth and highly decreased the competitive strength of all three varieties. Summer grazing decreased the lucerne proportion more than spring grazing. Camporegio seemed to have the lowest and Verbena the highest competitive strength (Table 3). The varieties were less resistant to grazing than had been reported by Pecetti *et al.* (2008).

Plant density differed between varieties. In spring there was, on average, a distance of 4.1 cm from a random point to the nearest lucerne plant for Verbena, 5.2 cm for Camporesio and 4.4 cm for Luzelle. Thus, the plant density was slightly lower for Camporegio (P < 0.05). In autumn Verbena had the most dense plant population and Camporegio the least (Table 4). There were, however, no significant differences between Camporegio and Luzelle.

	Cut 1 (C-G-C)				Cut 2 (G-C-G)				Cut 3 (C-G-C)			
	LU	GR	WC	OUS	LU	GR	WC	UP	LU	GR	WC	OUS
Verbena	74.7ª	19.6	2.4 <sup>b</sup>	3.3 <sup>b</sup>	66.5ª	23.6	5.8 <sup>b</sup>	4.1	21.3ª	36.1	16.2	26.5
Camporegio	63.1 <sup>b</sup>	25.3	5.6 <sup>a</sup>	6.0 <sup>a</sup>	51.0 <sup>b</sup>	30.5	13.3ª	5.1	11.4 <sup>b</sup>	37.5	21.3	29.8
Luzelle	74.5ª	19.4	3.2 <sup>ab</sup>	3.9 <sup>ab</sup>	58.6 <sup>ab</sup>	32.0	5.8 <sup>b</sup>	3.6	14.2 <sup>b</sup>	40.4	16.5	28.9
10L/15G	56.1°	34.9ª	4.7	4.3	48.0 <sup>b</sup>	41.3	8.2	2.5 <sup>b</sup>	8.1°	48.4	19.6	23.8 <sup>b</sup>
15L/10G	70.1 <sup>b</sup>	22.3 <sup>b</sup>	3.5	4.2	54.2 <sup>b</sup>	35.2	7.9	2.7 <sup>b</sup>	14.0 <sup>b</sup>	52.9	14.1	19.0 <sup>b</sup>
25L/0G	92.5ª		2.7	4.9	81.5ª		9.1	9.5ª	29.5ª		21.3	49.2ª

Table 3. Botanical composition in the tree cuts. Lucerne (LU), grass (GR), unsown white clover (WC) and other unsown species (OUS)

Different letters indicate significant differences (P < 0.05) between varieties or seed mixtures within each column.

There were tendencies to a more dense plant population after summer than after spring and autumn grazing, but no significant differences were found. Thus, the high reduction in lucerne proportion in cut 3 was reflected in a significantly lower plant density. Verbena had the highest lucerne proportion at cut 3 (21.3% in Table 3) and at the same time the highest plant density just after cut 3 (4.4 cm in Table 4). For Verbena there was only a decrease from 4.1 to 4.4 cm in management C-G-C, although there was a high decrease in growth and lucerne proportion. The plant density was not reduced as much as expected from the changes in botanical composition. A subsequent management that could pay regards to this could possibly have improved the lucerne plants again.

Table 4. Plant density in autumn, in late October after cut 3, measured as the distance from a random point to the nearest lucerne plant (cm)

Mixture	C-G-C	G-C-G	Variety	C-G-C	G-C-G
10 L/15 G	7.5 <sup>aA</sup>	9.7ªA	Verbena	4.4 <sup>bA</sup>	6.2 <sup>bA</sup>
15 L/10 G	6.6 <sup>aA</sup>	9.6 <sup>aA</sup>	Camporegio	8.1 <sup>aA</sup>	10.6ªA
25 L/0 G	4.9 <sup>bA</sup>	4.2 <sup>bA</sup>	Luzelle	7.8 <sup>aA</sup>	9.9 <sup>abA</sup>

Different letters indicate significant results (P < 0.05). Lower-case letters within mixtures and varieties, respectively, and upper-case letters within different managements.

## Conclusions

The two new grazing-tolerant varieties Verbena and Camporegio did not show conclusive differences to the older variety Luzelle. In relation to growth and competitive strength, Verbena performed best, Luzelle intermediate and Camporegio lowest. However, there is a need for further studies on the effect of grazing/cutting systems and grazing pressure to evaluate the possibility for improving the persistence.

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## References

Pecetti L., Romani M., De Rosa L. and Piano E. (2008) Selection of grazing-tolerant Lucerne cultivars. *Grass and Forage Science* 63, 360–368.

Søegaard K. and Møller K. (2005) White clover soil fatigue: an establishment problem on large and intensive dairy farms. In J.J.Murphy. *Proceedings of a satellite workshop of the XXth International Grassland Congress*, July 2005, Cork, Ireland, p. 231.

Søegaard K. and Weisbjerg M.R. (2007) Herbage quality and competitiveness of grassland legumes in mixed swards. *Grassland Science in Europe* 12, 166–169.

# Forage yield and quality of pasture mixtures in organic farming

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## Abstract

A study was carried out in 2007–2009 which aimed to evaluate the yield and chemical composition of pasture mixtures cultivated in organic farming. In the scheme of the experiment, the first factor was dose of natural fertilization (5, 10, 15 t manure per ha) and the second factor was percentage of legume seeds in mixtures with grasses (20, 35, 50%). Results showed that increased dose of organic fertilization caused significant increase of dry matter yield of mixtures. The chemical composition of fodder was dependent on the dose of organic fertilization, but this was generally satisfactory in terms of ruminant requirements demand. Increasing the dose of organic fertilization caused an increase of total protein content, crude fat, crude ash, and a slight decrease of crude fibre in the tested mixtures. Forage concentrations of major macro-elements were optimal.

Keywords: pasture mixtures, organic farming, manure, yield, chemical composition

## Introduction

For organic farming with dairy cattle, it is important to provide sufficient quantities of nutritional feed. The main source of the feed should be permanent pasture with legume plants, which allow reduced use of nitrogen (N) fertilizers and also contribute to reducing the costs associated with the production of feeds. According to Goliński (2008) the presence of legumes at 20–40% in a grass sward ensures levels of productivity similar to sown grass fertilized at 180 kg N ha<sup>-1</sup>. Nutrient-rich green forage, and properly prepared silage and hay, are the perfect alternatives to concentrated feed, which is particularly important in terms of ecological management (Jankowska-Huflejt *et al.*, 2006). Introduction to grasslands of perennial fodder-crop species that are valuable in terms of nutrition has a significant impact on the quantity and quality of the generated feed. The aim of the studies was to evaluate the yield and forage quality of pasture mixtures with different proportions of components in different fertilizing conditions of organic farming.

## Materials and methods

A large-area experiment was conducted in 2007–2009 at the Agricultural Experimental Station of IUNG, Grabów (51°21' N; 21°40' E), on permanent grassland renovated by full cultivation. The pasture mixtures were sown on the grey-brown podsolic soil belonging to a very good rye complex. The soil reaction was neutral, the mean content of available nutrients (mg per 1000 g soil) was: P - 306 mg, K - 114 mg, Mg - 374 mg. In the scheme of the experiment two factors were included: the dose of organic fertilization (5, 10, and 15 t composted manure per ha) and the percentage of legume seeds in mixtures: 20, 35, 50%, in relation to the mass of seeds sown in the pure sowing. Mixtures comprised the following species: *Lolium multiflorum*, *L. perenne*, *Festuca pratensis*, *F. rubra*, *Poa pratensis*, *Dactylis glomerata*, *Phleum pratense*, *Trifolium repens*, *T. pratense* and *T. hybridum*. The experiment was conducted in a split-block design, with two replications. The plot area was 720 m<sup>2</sup> (at harvest 15 m<sup>2</sup>). A grazing with dairy cows was conducted in the year of sowing, and in both years of utilization the mixtures were used in mown-grazing system. The dry matter (DM) yields and chemical composition of plants

were determined. Assessment of significance of the impact of the considered factors on the features under investigation was based on the variance analysis, indicating Tukey's confidence half-intervals at a significance level of 0.05.

## **Results and discussion**

The results of the studies showed that the level of fertilizing with compost manure was an important factor affecting the yields of forage, and increasing the dose of manure resulted in increased DM yield of mixtures. However, important differences in yields in both years of utilization and total yield were observed (Table 1). In the first year of utilization, the DM yield of mixtures fertilized with 10 t ha<sup>-1</sup> manure was approximately 10%, and for those fertilized with 15 t ha<sup>-1</sup> it was approximately 26% higher than the yield of mixtures fertilized with 5 t ha<sup>-1</sup>. In the second year of utilization these differences were 3% and 22%, respectively.

Table 1. Yield of dry matter depending on the dose of fertilization and share of legume seeds in mixtures (t  $ha^{-1}$ )

Specifi	antiona		Total vialda		
Specifi	cations	Sowing year	1st year of utilization	2 <sup>nd</sup> year of utilization	Total yields
Dose of organic fertilization	5	1.74	9.07	10.04	20.85
	10	1.87	10.00	10.32	22.19
	15	1.50	11.43	12.24	25.17
(t na ·)	LSD, $P = 0.05$	ns	1.251	0.752	1.254
Shara of logumo	20	1.62	9.80	10.88	22.38
seeds in mixture (%)	35	1.75	10.26	11.01	23.19
	50	1.74	10.44	10.70	22.88
	LSD, $P = 0.05$	ns	0.323	ns	ns

Different proportions of legume seeds in a mixture only slightly affected the level of DM yield. Only in the first year of utilization were there significantly increased yields of DM from the mixture with 50% of legumes, in comparison with the seed mixture with 20% of legumes. However, comparing the total yields, statistical differences were not found. Harasim (2008), in his earlier research showed that an increase of the percentage of clover in a mixture from 20 to 40% caused a significant increase in total yield, but further increase in the share of legume component (75%) caused a decrease in yield.

Vegeta- tion years	Dose of organ- ic fertilization (t ha <sup>-1</sup> )	Total protein	Crude fibre	Crude fat	Crude ash	Р	К	Ca	Mg	Na
	5	180.6	223.5	33.6	88.0	4.1	17.5	16.8	2.5	1.4
Coursing was	10	179.4	228.6	41.4	83.5	4.3	16.4	15.5	2.6	2.1
Sowing year	15	192.5	205.4	52.1	87.6	4.4	18.6	15.4	3.3	2.4
	LSD, $P = 0.05$	ns	18.37	6.89	ns	ns	ns	ns	ns	ns
The first year of	5	177.5	210.3	48.0	84.6	2.4	17.1	17.5	2.1	_
	10	183.5	223.3	45.4	86.8	2.7	19.0	18.0	3.0	-
	15	190.0	228.9	43.8	88.6	3.0	20.0	17.3	3.8	-
utilization	LSD, $P = 0.05$	ns	ns	ns	ns	0.37	2.46	ns	0.75	
The second	5	171.8	223.0	36.2	76.2	2.6	18.0	14.0	2.1	0.6
The second	10	186.3	220.1	42.3	82.7	2.7	19.1	14.5	2.6	0.7
year or	15	184.3	223.5	37.9	98.2	2.9	19.3	14.1	3.3	1.2
utilization	LSD, $P = 0.05$	15.03	ns	2.86	ns	ns	ns	ns	0.51	0.32
	5	176.6	218.9	39.3	82.9	3.0	17.5	16.1	2.2	1.0
Moon	10	183.1	224.0	43.1	84.3	3.2	18.2	16.0	2.7	1.4
Mean	15	188.9	219.3	44.6	91.5	3.5	19.3	15.6	3.4	1.8
	LSD, $P = 0.05$	ns	ns	2.29	ns	0.31	ns	ns	0.78	0.48

Table 2. Chemical composition of dry matter yield depending on the dose of organic fertilization (g kg<sup>-1</sup>)

The chemical composition of the pasture mixtures depended mainly on the quantity of manure. Together with the increase of the dose, there was an increase in the average protein content, crude fat and crude ash, and in concentrations of P, K and Mg in the DM, but there was a decrease in crude fibre content (Table 2). There was no influence of the dose of fertilizer on the content of Ca and Na. Natural fertilizers are undoubtedly an important source of macro- and micro-nutrients for fodder plants. An increase in the protein content under the influence of fertilizing manure was reported by Bojarszczuk *et al.* (2011). Different proportions of legume seeds in a mixture did not have a significant influence on the content of basic nutrients and of macro-elements in the feed from mixtures tested here (Table 3). Only in the year of sowing was a higher total protein content, and a lower content of crude fibre in dry mass of mixtures, found to occur from the mixture with the largest proportion of clover (50%).

Vegeta- tion years	Share of legume seeds in mixture (%)	Total protein	Crude fibre	Crude fat	Crude ash	Р	К	Ca	Mg	Na
	20	177.1	222.4	38.4	85.2	4.5	17.6	15.5	2.9	1.8
C	35	179.4	222.5	45.7	87.5	4.2	18.0	15.4	2.5	2.3
Sowing year	50	196.1	212.6	43.1	86.4	4.1	16.9	16.7	3.0	1.9
	LSD, $P = 0.05$	ns	ns	6.89	ns	ns	ns	ns	ns	ns
The first	20	183.1	221.1	44.5	87.1	2.8	18.7	17.7	3.0	_
The first	35	186.7	220.8	46.5	89.5	2.8	19.4	18.0	2.8	-
year or	50	181.0	220.6	46.2	83.5	2.7	17.9	17.1	3.1	_
utilization	LSD, $P = 0.05$	ns	ns	ns	ns	ns	ns	ns	ns	
The second	20	180.4	217.5	37.4	92.9	2.7	18.9	14.5	2.7	0.8
The second	35	180.4	225.1	39.0	85.1	2.7	19.9	14.4	2.5	0.9
year or	50	181.6	223.9	40.1	79.1	2.8	17.5	13.7	2.9	0.8
utilization	LSD, $P = 0.05$	ns	ns	ns	ns	ns	ns	ns	ns	ns
	20	180.2	220.3	40.1	88.4	3.3	18.4	15.9	2.9	1.3
	35	182.2	222.8	43.7	87.4	3.2	19.1	15.9	2.6	1.6
wiean	50	186.2	210.0	43.1	83.0	3.2	17.4	15.8	3.0	1.4
	$I_{SD} P = 0.05$	ns	ns	2 29	ns	ns	ns	ns	ns	ns

Table 3. Chemical composition of dry matter yield depending on the share of legume seeds in mixtures (g  $kg^{-1}$ )

## Conclusions

Variability of the dry matter yield of pasture mixtures was more dependent on the dose of manure than on the proportion of legume seeds sown. There was a significant increase in yield with increased rate of manure application. The content of basic nutrients in the obtained feed was mainly dependent on the dose of manure, and only slightly affected by the share of legumes in the pasture. Increasing the dose of manure caused an increase in the content of total protein, crude fat and crude ash, while the content of crude fibre slightly decreased. In terms of ruminant nutritional requirements, the obtained animal feed had an optimal content of P, K, Mg, but Na content was slightly lower than the desired content, and Ca content was too high.

## References

Bojarszczuk J., Staniak M. and Harasim J. (2011) Productivity of pasture mixtures with legumes in organic farming. *Journal of Research and Applications in Agricultural Engineering* 56(3), 27–35 (in Polish with English Summary).

Goliński P. (2008) Current trends in production technologies of fodder plant raw materials. *Pamiętnik Puławski* 147, 67–82 (in Polish with English summary).

Harasim J. (2008) Yielding of pasture sward with white clover in relation to the seeding rates and habitat. *Woda Środowisko Obszary Wiejskie* 8(2b), 19–29 (in Polish with English summary).

Jankowska-Huflejt H., Wróbel B. and Kaca E. (2006) Utilization and quality of fodders from grasslands in organic livestock production in Poland. Proceedings of European Joint Organic Congress Organic Farming and European Rural Development, 30–31 May 2006 in Odense, Denmark, pp. 456–457.

# Effects of daylength and soil compaction on white clover growth and N<sub>2</sub>- fixation

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## Abstract

In order to detect the efficiency of the nitrogen ( $N_2$ ) fixation in clover-grass leys in northern climate conditions, we studied how soil compaction affects growth and  $N_2$ -fixation of white clover (*Trifolium repens* L.) under contrasting growth conditions. A pot experiment was carried out under controlled climatic conditions in the phytotron at Holt (Tromsø). Sandy soil was compacted to two levels, 60% and 85% of the standard degree of compactness (SDC). Four seedlings of white clover plants or timothy (*Phleum pratense* L.) were carefully planted in each pot. Timothy was used as reference plant. The plants were placed at 15°C for twelve weeks and subjected to 18 or 24 h daylight. The <sup>15</sup>N isotope dilution method was used to assess  $N_2$ -fixation. Results suggest that 24 h daylight increased white clover biomass production as compared to 18 h daylight and favoured leaf and stolon production significantly more at 85% of SDC than at 60% of SDC. However, for white clover plants grown at 18 h day-length, higher compactness reduced the root development. On average, white clover derived 44–58% of its total N from  $N_2$ -fixation grown at 60% of SDC and 46–47% at 85% of SDC, regardless of light conditions. The  $N_2$ -fixation was somewhat higher at 24 h day length only under the low soil compaction level.

Keywords: controlled environment, light, nitrogen fixation, sandy soil, Trifolium repens

## Introduction

Clovers are crucial for N supply in low-input farming systems. About 80% of clover N is derived from the atmospheric N (Huss-Danell *et al.*, 2007). Frame and Newbould (1986) observed that active N<sub>2</sub>-fixation requires a minimum soil temperature of about 9°C. In northern latitudes the growing season is short and cool. However, the light conditions may, to a certain degree, counteract the negative effects of low temperature due to positive effects on biomass production. Clovers are also vulnerable to adverse soil conditions. The development of modern farming systems has resulted in more frequent use of heavier farm machinery. Thus, tractor traffic may negatively affect yield of forage crops, either by directly damaging the above-ground plant parts or by soil compaction. The objective of the present work (funded by the Research Council of Norway) was to investigate effects of day-length and soil compaction on white clover growth and N<sub>2</sub>-fixation under controlled climatic conditions.

## Materials and methods

A pot experiment was conducted in the phytotron at the University of Tromsø. The treatments comprised the two plant species [white clover (*Trifolium repens* L.) and timothy (*Phleum pratense* L.) as a reference plant], two soil compaction levels and two day-length regimes. The experimental design was a split-plot factorial with three replicates. Soil used in the pot experiment was collected from the 0–20 cm layer of an organically cultivated field from Tjøtta in the

county of Nordland (65°49'N, 12°25'E). The soil pH was 6.5, and consisted of 86% sand, 11% silt and 3% clay. It was sieved through a 6 mm mesh. The soil was stored moist and dark at 2°C until it was potted. Soil water content was 22.4%. At the start of the trial the soil was compacted to two different levels with the aim of obtaining a relative degree of compactness of 60% (RDC<sub>85%</sub>) or 85% (RDC<sub>85%</sub>) of its standard degree of compactness (SDC). RDC has been previously used in Scandinavian agricultural research (Kristoffersen and Riley, 2005). The SDC was estimated for the collected soil using equation 1.1.

 $SDC = 1.751 - 0.032 \times ignition - loss (\%) - 0.0032 \times silt (\%) + 0.0065 \times gravel (\%) + 0.0029 \times clay (\%)$ [1.1]

To obtain 85% RDC, a hydraulic press was used to compact 3.5 kg of soil to the desired volume (2.5L) whilst for 60% RDC 2.5 kg of soil was compacted by hand. The <sup>15</sup>N isotope dilution technique (ID) was used to estimate N fixation by white clover. <sup>15</sup>N fertiliser (0.094 g 10% <sup>15</sup>NH<sub>4</sub>Cl per pot) was uniformly applied in a liquid form to all pots except control pots that received distilled water. About 10 g of soil was then removed from each pot to measure total N. Four seedlings of white clover and timothy plants (seeds were put for germination on moist filter two weeks before the start of the experiment) were then gently planted in each pot. The experiment was carried out at 15°C with 18 h or 24 h photoperiod representing growing conditions typical for northern Norway. The experiment lasted 12 weeks and during this time plants were watered with solution containing K, P, Ca, Mg and trace elements to meet plant growth requirements. Afterwards plants were harvested. About 50 g of soil, representative of each pot, was collected before roots were washed free of soil over 3 mm mesh. The white clover plants were then separated into leaves, stolons and roots, and timothy plants into leaves and roots. Dried plant samples (60°C, 48 h) were weighed, ground and analysed for total N and <sup>15</sup>N enrichment. Concentrations of total N and <sup>15</sup>N were determined using elemental analyser-isotope ratio mass spectrometry. Data on <sup>15</sup>N enrichment of biomass were used to calculate the percentage of clover N derived from symbiotic  $N_2$ -fixation (% $N_{dfa}$ ) according equation 1.2

 $%N_{dfa} = 1 - (15N \text{ atom}\% \text{ excess in clover}/15N \text{ atom}\% \text{ excess in timothy}) \times 100$  [1.2] Analysis of variance (General Linear Model) was used to test significance of differences among the various treatments.

#### **Results and discussion**

There were significant interactions between the effects of soil compaction level and day length on leaf, stolon and root development. Under 18-h light conditions, soil compaction reduced root growth by about 50% whilst development of shoots and stolons was not affected (Figure 1A). At 24 h day-length, white clover produced significantly larger aboveground biomass at RDC<sub>85%</sub> than at RDC<sub>60%</sub> (P < 0.005) whilst the root production was almost similar (Figure 1B). It was somewhat surprising that the higher compaction level resulted in greater above-ground biomass production. Håkonsson et al. (1990) concluded that for plant growth the optimal range of RDC is 81–89% of SDC. For sandy soil RDC<sub>sour</sub> was, probably, too low. It seems that the coarse soil texture and large pores favoured root development, rather than growth of leaves and stolons. These results indicate that for sandy soils the soil compaction level at RDC<sub>85%</sub> has relatively little effect on white clover growth. There were no significant differences on proportion of N derived from N<sub>2</sub>- fixation between treatments. The  $N_{dfa}$  was generally low (Figure 2). On average, white clover derived 44–58% of its total N from N<sub>2</sub>-fixation when grown at RDC<sub>60%</sub> and 46–47% at RDC<sub>85%</sub>. The  $N_2$ -fixation was somewhat higher at 24 h day-length, but only for white clover plants grown at level of low soil compactness.



Figure 1. Mean dry matter (g  $pot^{-1}$ ) of white clover leaves, stolons and roots at two levels of soil compaction and at 18 h day length (A) and 24 h day length (B)



 $N_2$  fixation (% $N_{dfa}$ ) in white clover plants at two levels of soil compaction and two different day-lengths

## Conclusions

The high level of soil compaction reduced white clover root development by half, but only at 18 h day-length. The development of leaves and stolons was unaffected regardless on day-length. This indicated that sandy soils are less susceptible to soil compaction and are able to maintain white clover growth under such conditions. The  $N_{dfa}$  was low, on average 49%, for all treatments.

#### References

Huss-Danell K., Chaia E. and Carlsson G. (2007)  $N_2$  fixation and nitrogen allocation to above and below ground plant parts in red clover-grasslands. *Plant and Soil* 299, 215–226.

Håkansson I., Voorhees W.B. and Riley H. (1988) Vehicle and wheel factors influencing soil compaction and crop response in different traffic regimes. *Soil Tillage Research* 11, 239–282.

Frame J. and Newbould P. (1986) Agronomy of white clover. Advances in Agronomy 40, 1-88.

Kristoffersen A.Ø. and Riley H. (2005) Effects of soil compaction and moisture regime on the root and shoot growth and phosphorus uptake of burley plants growing on soils with varying phosphorus status. *Nutrient Cycling in Agroecosystems* 72, 135–146.

# Earliness as a means of designing seed-mixtures, as illustrated by *Lolium* perenne and Dactylis glomerata

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## Abstract

In mixtures containing *Lolium perenne* and *Dactylis glomerata*, *L. perenne* is outcompeted over time by the more competitive *D. glomerata*. However, due to its superior forage quality a high proportion of *L. perenne* is desirable. Dry matter (DM) yield, botanical composition, digestibility and net-energy in the third growing-year have been assessed in grass-clover mixtures containing either two early or two late *L. perenne* cultivars combined with different cultivars of *D. glomerata*. Whole-sward annual DM yield (13.9 Mg ha<sup>-1</sup>) was not affected. Also, the cultivars of *D. glomerata* did not affect any of the traits. However, mixtures with early flowering *L. perenne* showed a five-times higher proportion (30%) of *L. perenne* than mixtures with late cultivars (P < 0.01). The higher proportion of *L. perenne* led to an increase in digestibility from 673 to 693 g kg<sup>-1</sup> DM (P < 0.05) and in net-energy from 5.95 to 6.13 MJ NEL kg<sup>-1</sup> DM (P < 0.05). This increase in energy translates into a gain in annual milk production of about 1 Mg Milk ha<sup>-1</sup>. The results show that the earliness of cultivars offers opportunities when designing seed mixtures.

Keywords: mixtures, Lolium perenne, Dactylis glomerata, forage quality, yield

## Introduction

In comparison to grass monocultures, grass-legume mixtures offer many benefits: they are more productive (Lüscher *et al.*, 2008; Nyfeler *et al.*, 2009), provide important amounts of nitrogen to the plant-soil-system (Nyfeler *et al.*, 2011), increase forage intake (Rochon *et al.*, 2004), and make it possible to extend the harvesting period without compromising forage quality (Peyraud *et al.*, 2009). The ease of combining different forage plant species allows design of mixtures suited to specific environmental conditions and utilisation systems (Suter *et al.*, 2008b). The botanical composition of a grass-legume mixture and, thus, its

			-						
			kg seed ha <sup>-1</sup>						
Species		cultivar	Dg cv.	Pizza	Dg cv. Baraula				
-			'early'	'late'	'early'	'late'			
Trifolium pratense L.		Merviot	2.0	2.0	2.0	2.0			
Trifolium repens L.		Milo	1.5	1.5	1.5	1.5			
		Seminole	2.5	2.5	2.5	2.5			
Lolium perenne L.	'early'	Arvicola	3.0	_	3.0	_			
-	-	Alligator	4.0	_	4.0	_			
	'late'	Anaconda	_	3.5	_	3.5			
		Elgon	_	3.5	_	3.5			
Dactylis glomerata L.		Pizza	5.5	5.5	_	_			
		Baraula	_	_	5.5	5.5			
Festuca pratensis L.		Préval	12.0	12.0	12.0	12.0			
Phleum pratense L.		Tiller	2.5	2.5	2.5	2.5			
Total			33.0	33.0	33.0	33.0			

Table 1. Composition (kg seed ha<sup>-1</sup>) of mixtures with 'early' and 'late' sets of cultivars of *Lolium perenne* L., respectively, and two cultivars of *Dactylis glomerata* L. (DG)

function, is largely determined by interspecific competition. Since the competitive ability may vary greatly among cultivars of a plant species (Suter *et al.*, 2007; Suter *et al.*, 2008a), the choice of cultivars may influence the botanical composition of the mixture (Suter *et al.*, 2007). In mixtures containing *Lolium perenne* and *Dactylis glomerata*, the *L. perenne* is usually outcompeted over time by the less digestible *D. glomerata* (Nyfeler *et al.*, 2009). Thus, there is an urgent need for more competitive cultivars of *L. perenne* and it is of great interest to determine:

i) how earliness of *L. perenne* cultivars in grass-legume mixtures containing *D. glomerata* affects the botanical composition in the third year, and;

ii) to what extent dry matter (DM) yield and forage quality of the mixtures are altered by using early *L. perenne* cultivars instead of late ones.

## Materials and methods

Four variants of the grass-legume mixture, 'SM330' (Suter *et al.*, 2008b) were sown at the Agroscope Reckenholz-Tänikon Research Station ART in Zurich-Reckenholz in spring 2003. The composition of the mixtures is shown in Table 1. Four cultivars of *L. perenne* L. (LP) were used in order to specify two sets: an 'early' set (cv. Arvicola and cv. Alligator) and a 'late' set (cv. Anaconda and cv. Elgon), with a difference in heading date of the inflorescences of about 10 days. Two cultivars of *D. glomerata* L. (DG) that did not differ in earliness (cv. Pizza and cv. Baraula) were used in order to broaden the basis of the test. All mixtures were mown five times per year and fertilised with 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>. In the third year of the experiment the mean DM proportions of *L. perenne* were recorded by manually separating plant samples. Additionally, annual DM yield and digestibility were measured. Digestibility, expressed as digestible organic matter (DOM) was analysed by near-infrared spectroscopy (NIRS) and validated with an *in vitro* technique according to Tilley and Terry (1963). Net energy lactation (NEL) was calculated according to RAP (1999). Data were analysed by analysis of variance, testing 'LP,' 'DG' and 'LP × DG' interaction effects.

## **Results and discussion**

Mixtures with the 'early' set showed a five-times higher proportion (P < 0.001) of *L. perenne* (30%) than mixtures with 'late' cultivars (Figure 1a). This result can be interpreted as an effect of a better competitive ability of 'early' cultivars compared to 'late' cultivars. In this experiment the effect may have been especially strong because the heading of inflorescences of the 'early' set was three days earlier than that of the DG-cultivars (Suter *et al.*, 2008a), and that of the 'late' set was yet another seven days later (Suter *et al.*, 2008a). In addition to the direct effect of earliness, i.e. plant development, the better persistence and competitive ability of early flowering cultivars, as suggested by data from 28 cultivars grown in monocultures (Suter *et al.*, 2006), may have influenced the performance of the LP cultivars. Aside from the shift from DG-dominated swards toward compositions with a greater proportion of LP at the expense of DG, the proportions of the other plant species were unchanged (data not shown).

In contrast to LP proportion, DM yield (Figure 1b) was not affected by any of the two factors. It remained at 13.9 Mg ha<sup>-1</sup> which is, under typical conditions in Switzerland, regarded as a good yield. In mixtures with DG, replacing 'late' LP with 'early' cultivars resulted in a significant (P < 0.05) increase in digestibility from 673 to 693 g kg<sup>-1</sup> dry matter (Figure 1c). Although this difference in DOM is rather small, it led to a significant (P < 0.05) improvement in the content of NEL from 5.95 to 6.13 MJ NEL kg<sup>-1</sup> DM (Figure 1d). For such an increase in energy content and a DM yield of 13.9 Mg ha<sup>-1</sup>, an increase in annual milk yield of roughly 1 Mg ha<sup>-1</sup> can be calculated using the formulae of RAP (1999).



Figure 1. a) Proportion of *Lolium perenne* L., b) DM Yield, c) digestible organic matter (DOM), and d) net energy lactation (NEL) of mixtures with 'late' (open bars) and 'early' (solid bars) sets of cultivars of *L. perenne* (LP) and two cultivars of *Dactylis glomerata* L. (DG). Statistical tests: ns = non significant, \* P < 0.05, \*\*\* P < 0.001

#### Conclusions

We conclude that earliness of *L. perenne* cultivars used in mixtures containing *D. glomerata* has an effect on botanical composition of the plant stand and on quality of the forage. Therefore, we suggest that the concept of considering earliness may offer important options for designing high-quality seed mixtures in general.

#### References

Lüscher A., Finn J.A., Connolly J., Sebastià M.T., Collins R., Fothergill M., Porqueddu C., Brophy C., Huguenin-Elie O., Kirwan L., Nyfeler D. and Helgadóttir Á. (2008) Benefits of sward diversity for agricultural grasslands. *Biodiversity* 9, 29–32.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Lüscher A. (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology* 46, 683–691.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E. and Lüscher A. (2011) Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems and Environment* 140, 155–163.

Peyraud J.L., Le Gall A. and Lüscher A. (2009) Potential food production from forage legume-based systems in Europe: an overview. *Proceedings of International Conference, Forage legumes in temperate pasture-based systems, Cork, Ireland,* 1–22.

RAP (1999) Fütterungsempfehlungen und Nährwerttabellen für Wiederkäuer, LMZ, Zollikofen, CH, 328 pp. Rochon J.J., Doyle C.J., Greef J.M., Hopkins A., Molle G., Sitzia M., Scholefield D. and Smith C.J. (2004) Grazing legumes in Europe: a review of their status, management, benefits, research needs and future prospects. *Grass and Forage Science* 59, 197–214.

Suter D., Briner H.U. and Lüscher A. (2007) Effect of *Lolium perenne* L. variety on the botanical composition of grass-clover mixtures. *Grassland Science in Europe* 12, 417–420.

Suter D., Briner H.U., Rosenberg E., Mosimann E., Jeangros B., Bertola C. and Stévenin L. (2006) Englisches Raigras: 61 Sorten geprüft. *Agrarforschung* 13 (7), 278–283.

Suter D., Hirschi H.U., Briner H.U., Frick R., Jeangros B. and Bertossa M. (2008a) Liste der empfohlenen Sorten von Futterpflanzen 2009–2010. Agrarforschung 15 (10), I–VIII.

Suter D., Rosenberg E., Frick R. and Mosimann E. (2008b) Standardmischungen für den Futterbau: Revision 2009–2012. *Agrarforschung* 15(10), 1–12.

Tilley J. and Terry R. (1963) A two stage technique for the in vitro digestion of forage crops. *Journal of the British Grassland Society* 18, 104–111.

# The effect of nitrogen fertilization on quality and yield of grass-legume mixtures

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## Abstract

Studies were carried out in pure stands of three grasses: cocksfoot, meadow fescue and perennial ryegrass, and lucerne, as well as their mixtures with different ratios of individual components. The trial was carried out on an experimental field of the Institute for Animal Husbandry (Belgrade, Serbia). Fertilization with N caused a significant increase of yield. The highest yields were determined in treatments with 100 kg N ha<sup>-1</sup>, and the lowest in treatments without fertilization. Fertilization increased significantly the CP content, but without impact on other quality parameters. CP, NDF and ADF differed significantly in various crops.

Keywords: grass-legume mixture, quality, dry matter yield

# Introduction

Grass-legume mixtures, as well as pure stands, are important for production of high quality forage, especially in conditions of lowland livestock production, in conditions of farm housing and systems of free grazing of animals. Growing of lucerne and other legumes in mixtures with grasses has numerous advantages, such as a possibility of utilization through grazing, better conservation of water and mineral salts in soil (Lazaridou et al., 2006), higher yields than pure stands during vegetation, better quality of forage, better utilization of soil nutrients, as well as more economically efficient production due to reduced use of nitrogen fertilizers (Tekeli and Ates, 2005). Applied nitrogen fertilizers in a grass-legume mixture increase the DM yield, as well as the content and yield of CP. Komarek et al. (2007), in their study of the effect of complex mineral fertilizers, with and without N, on yield of grasslands, concluded that by adding 90 kg N ha<sup>-1</sup> the yield of DM increased by 1.89 t ha<sup>-1</sup>, and by adding 180 kg N ha<sup>-1</sup> an increase of 3.03 t ha<sup>-1</sup> was recorded. In the study by Tomić et al. (2011), fertilization increased significantly the yield of CP by 194.1 kg ha<sup>-1</sup> in monoculture and by 323.2 kg ha<sup>-1</sup> in mixtures. The objective of this research was to study the effect of different N rates from mineral fertilizers on production and qualitative properties of grasses and legume plants in monoculture and in mixtures.

## Material and methods

The trial was carried out on the experimental field of the Institute for Animal Husbandry, according to a CRB design in 4 replicates. The effect of 3 N rates (0, 50 and 100 kg ha<sup>-1</sup>) on DM yield, content of CP, CF, NDF and ADF in pure crops (lucerne, cocksfoot, meadow fescue and perennial ryegrass) and grass-legume mixtures: lucerne and cocksfoot (50:50); lucerne, cocksfoot and meadow fescue (50:25:25 and 25:50:25); lucerne, cocksfoot, meadow fescue and perennial ryegrass (40:20:20:20), was investigated. Sowing was carried out in the autumn of 2010, on basic parcel/plot of 10 m<sup>2</sup>. Phosphorus was deficient and it was added at the amount of 165 kg ha<sup>-1</sup>. N fertilization of crops was carried out by the end of March 2011, using 27% N. Soil type was moderate humus and slightly or moderately acid. Results of total yield of DM in pure crops and grass-legume mixtures, as well as of the quality in the first cut, were processed using standard laboratory methods and analysed

by parametric tests (ANOVA and LSD test) at the 0.05 probability levels, using statistical package Statistica 8.

## **Results and discussion**

Based on the results obtained (Table 1), fertilization with  $0-100 \text{ kg N} \text{ ha}^{-1}$  caused a significant increase in yield of pure crops and grass-legume mixtures by an average of 1.42 t ha<sup>-1</sup>. In pure crops, the highest yield of 7.66 t ha<sup>-1</sup> was recorded in perennial ryegrass fertilized with the highest N rate, whereas the lowest yield was obtained in the control treatment of cocksfoot (4.94 t ha<sup>-1</sup>). The highest yield (8.78 t ha<sup>-1</sup>) was realized by the four-component mixture in the treatment with 100 kg N ha<sup>-1</sup>, and the lowest (4.35 t ha<sup>-1</sup>) was for the mixture of lucerne, cocksfoot and meadow fescue (25:50:25) without fertilization. The results are consistent with findings of Bijelić *et al.* (2011) who concluded that DM yield increases in proportion to the amount of added N, 13.7–14.2 t ha<sup>-1</sup>.

Table 1. Total yield of DM of pure lucerne, cocksfoot, meadow fescue and perennial ryegrass crops and their mixtures, depending on the N fertilization ( $t ha^{-1}$ )

Crop	N fer	Means						
	0	50	100	mixture				
lucerne	6.13	6.62	7.01	6.59 <sup>b</sup>				
cocksfoot	4.94	5.84	5.80	5.53 <sup>cd</sup>				
meadow fescue	6.47	6.15	7.28	6.63 <sup>b</sup>				
perennial ryegrass	7.10	6.90	7.66	7.22ª				
lucerne+ cocksfoot 50:50	5.42	5.33	6.70	5.81°				
lucerne + cocksfoot + meadow fescue 50:25:25	5.91	7.51	7.00	6.80 <sup>b</sup>				
lucerne + cocksfoot + meadow fescue 25:50:25	4.35	6.60	7.22	6.05°				
lucerne + cocksfoot + meadow fescue + perennial rvegrass 40:20:20:20	5.70	7.62	8.78	7.36ª				
Means for N fertilization	5.75°	6.57 <sup>b</sup>	7.18 <sup>a</sup>	_				
$LSD_{out}$ mixture = 0.3830; $LSD_{out}$ fertilization = 0.3316								

 $\overline{\text{LSD}_{0.05}}$  – least significant difference at  $P \le 0.05$ .

In Table 2, the contents of CP, CF, NDF and ADF in pure crops and mixtures, depending on the N fertilization, are presented. CP was one of the most important parameters of forage quality, and it determines the biological value of food. Legumes are characterized by significantly higher CP content than in grasses. Conservation of forage in the form of hay can cause a decrease of CP content, because of improper handling, excessive drying or loss of leaves. Content of CP varied significantly in relation to crop and fertilization. The highest CP in pure crops was recorded in lucerne (14.9%), and in mixtures of lucerne and cocksfoot (12.68%). Fertilization influenced the increase CP of (9.90-13.10%). Komarek et al. (2007), reported that a treatment with the highest N rate (180 kg ha<sup>-1</sup>) influenced the increase of CP in grass-legume mixtures of 120.6–134.6 g kg<sup>-1</sup> DM. Content of CF in forage dry matter is an important energy parameter and significant component of every forage mixture. Studied factors had no significant influence (P = 0.08) of this quality parameter whose value ranged from 26.58–29.00%. Good balance of diet enables better intake of available food by animals. The ratio between ADF, NDF and fibre is especially important. In a diet for high yielding cows in lactation, it is necessary to ensure at least 19–21% ADF and 28-30% NDF. Roughage should ensure at least 21% NDF in DM. ADF and NDF do not change under the influence of fertilization of any mixtures or pure crops ( $P \le 0.05$ ), two indicators between different crops. Of all the factors studied, only sward type had an impact on NDF and ADF. The highest NDF was recorded in cocksfoot (63.59%) and in the mixture (25:50:25) of lucerne, cocksfoot and meadow fescue (63.56%), and the lowest in lucerne (44.48%) and the mixture of lucerne and cocksfoot (59.22%).

Main effect	СР	CF	NDF	ADF
Сгор				
lucerne	14.29	27.81	44.48	36.34
cocksfoot	11.10	28.78	63.59	37.44
meadow fescue	9.23	27.41	61.68	37.55
perennial ryegrass	8.94	26.58	52.69	32.57
lucerne+ cocksfoot 50:50	12.68	28.76	59.22	37.34
lucerne + cocksfoot + meadow fescue 50:25:25	13.08	29.00	61.30	36.31
lucerne + cocksfoot + meadow fescue 25:50:25	11.33	27.65	63.56	38.04
lucerne + cocksfoot + meadow fescue + peren-	12.20	20.28	60.05	20.10
nial ryegrass 40:20:20:20	12.29	29.28	00.93	39.19
LSD 0.05	1.24	ns	2.47	2.50
N fertilization (kg ha <sup>-1</sup> )				
0	9.90	28.11	57.61	36.84
50	11.86	28.45	58.57	37.08
100	13.10	27.91	59.11	36.62
LSD 0.05	1.07	ns	ns	ns

Table 2. CP, CF, NDF and ADF in pure crops and mixtures depending on fertilization (%)

Numerous research studies have reported that fertilization of grass-legume mixtures with N significantly increases the NDF, as a consequence of it increasing the proportion of grass, whereas ADF content remains unchanged under the influence of N (Bijelić *et al.*, 2011).

## Conclusions

In Serbia, grass-legume mixtures as well as pure stands of grassland species realized satisfactory yields of DM in the first production year, as well as quality: CP, CF, NDF and ADF. With an optimum rate of 100 kg N ha<sup>-1</sup>, the yield of pure stands of perennial ryegrass at 7.66 t DM ha<sup>-1</sup> was lower than the 8.78 t DM ha<sup>-1</sup> of 4-component mixtures with the same fertilization. This high quality mixture can be recommended for growing since it has significantly higher yield and better quality (CP, NDF, and especially ADF) in comparison with other studied mixtures.

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## References

Bijelić Z., Tomić Z. and Ružić-Muslić D. (2011) The effect of nitrogen fertilization on production and qualitative properties of sown grasslands in the system of sustainable production. *Biotechnology in Animal Husbandry* 27, 1, 615–631.

Komarek P., Nerušil P., Kohoutek A. and Odstrčilova V. (2007) The effect of repeated direct sowing of grasslegume seed mixtures into grasslands on forage production and quality. *Grassland Science in Europe* 12, 39–42. Lazaridou M., Vrahnakis M. and Noitsakis B. (2006) Performance of legume grass association in the field under drought and cutting conditions. http://www.fao.org/DOCREP/006/AD236E/ad236e0q.htm

Tekeli S.A. and Ates E. (2005) Yield potential and mineral composition of white clover (*Trifolium repens* L.) and tall fescue (*Festuca arundinacea* Schreb.) mixtures. *Journal of Central European Agriculture* 6, 1, 27–34.

Tomić Z., Bijelić Z., Žujović M., Simić A., Kresović M., Mandić V. and Marinkov G. (2011) Dry matter and protein yield of lucerne, cocksfoot, meadow fescue, perennial ryegrass and their mixtures under the influence of various doses of nitrogen fertilizer. *Biotechnology in Animal Husbandry* 27, 1, 1219–1226.

# **Productivity of wheatgrass** (*Agropyron cristatum* (L.) Gaertn.) as a component of pasture mixtures for the conditions of the Danube Plain

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## Abstract

In order to study wheatgrass (*Agropyron cristatum* (L.) Gaertn.) as a component of pasture mixtures for the Danube Plain region, Bulgaria, a field trial was carried out at the Institute of Forage Crops, Pleven in 2004–2010. Wheatgrass, cocksfoot, birdsfoot trefoil, sainfoin and white clover, in pure swards and simple double-mixtures of wheatgrass with perennial legumes, and wheatgrass with three legumes, were studied. Simple double-mixtures of cocksfoot with perennial legumes, three-component mixtures of wheatgrass and cocksfoot with legumes and five-component mixtures of grasses with all legumes were also studied. Dry matter yield from wheatgrass averaged over six years was 10% lower than that of cocksfoot. Wheatgrass was less productive than cocksfoot at the beginning (first and second cut) and more productive at the end of vegetation (third and fourth cut). Wheatgrass is a suitable component to establish persistent pasture mixtures with extensive type of use.

Keywords: wheatgrass, pasture mixtures, dry matter

## Introduction

Climate changes will have long-term effects on both the botanical composition and the dry matter yield of traditional grassland forage crops (Mannetje, 2006; Trnka *et al.*, 2011). Wheat-grass (*Agropyron cristatum* (L.) Gaertn.) is a relatively new species for Bulgaria and is characterized by drought tolerance and persistency (Katova, 2007). The aim of this work was to study the productivity of wheatgrass as a component of pasture mixtures for the conditions of the Danube Plain, Bulgaria.

## Materials and methods

A field trial (2004–2010) was carried out near the town of Pleven ( $43^{\circ}39'$  N;  $24^{\circ}58'$  E) Bulgaria under no irrigation with four replications and a plot size of 10 m<sup>2</sup>. Wheatgrass, cocksfoot, birdsfoot trefoil, sainfoin and white clover in pure swards and simple double-mixtures of wheatgrass with perennial legumes, as well as wheatgrass with three legumes, were tested. The simple double-mixtures of cocksfoot with perennial legumes, three-component mixtures of wheatgrass and cocksfoot with legumes and five-component mixtures of grasses with all legumes were also tested. The ratios in the mixtures were in accordance with the number of components and their sowing rate in the pure sward. No fertilizers were applied during the experimental period. The swards were grazed by sheep when they reached 18–20 cm (pasture maturity), and after grazing the residues were moved. Immediately before each grazing the dry matter (DM) yield was recorded (kg ha<sup>-1</sup>). Four cuts were taken from the plots every year (2005–2009), and during the final year (2010) there were three. The experimental data was analysed statistically using MS Excel, ANOVA LSD at *P* < 0.05.

## **Results and discussions**

The DM yield from the pure swards of cocksfoot exceeded those of wheatgrass significantly (Table 1). In the second year the productivity of all swards decreased. In the mixtures

with wheatgrass, where there were greater proportions of legumes, the decrease was 22% on average. In the third year, which was assessed as being very dry, a significant decrease of yield was recorded in all swards. There were no significant differences in the values of this characteristic between pure wheatgrass and cocksfoot.

Swonda	2005	2006	2007	2008	2009	2010	Average	annual yield
Swards				kg ha⁻	1			%
Wheatgrass	7843	6084	3164	2375	2426	3129	4170	100.0
Cocksfoot	11071	7270	2989	2309	1943	1858	4573	109.7
LSD 5%	1549	860	354	265	381	856	296	
Birdsfoot trefoil	12873	10936	5129	4783	3190	3565	6746	161.8
Sainfoin	13117	10749	4959	4506	2912	3424	6611	158.5
White clover	8935	8198	4779	5067	4493	2840	5719	137.1
Average	11641	9961	4956	4785	3532	3276	6359	
Wheatgrass + Birdsfoot trefoil	12393	10029	5246	4709	2807	3417	6434	154.3
Wheatgrass + Sainfoin	12689	9956	5083	4419	2439	3356	6324	151.6
Wheatgrass + White clover	8653	8019	3550	3158	2184	2984	4758	114.1
Wheatgrass + B. trefoil + Sainfoin+W. clover	12481	9983	4796	4681	2868	3520	6388	153.2
Average	11554	9497	4669	4242	2575	3319	5976	
LSD 5%	245	198	168	159	195	123	283	
Cocksfoot + Birdsfoot trefoil	12338	9156	5013	4963	3279	4125	6479	155.4
Cocksfoot + Sainfoin	12793	9015	4969	4851	2866	4446	6490	155.6
Cocksfoot + White clover	8865	8041	3096	4015	2544	3414	4996	119.8
Cocksfoot + B. trefoil + Sainfoin+W. clover	12358	8933	4728	4898	2880	4338	6356	152.4
Average	11589	8786	4451	4682	2892	4081	6080	
LSD 5%	315	262	315	224	186	286	246	
Wheatgrass + Cocksfoot + B. trefoil	12402	8789	4904	4862	2913	3393	6210	148.9
Wheatgrass + Cocksfoot + Sainfoin	12531	8936	4783	4793	2456	3118	6103	146.3
Wheatgrass + Cocksfoot + W. clover	8768	7984	2637	2186	1842	2972	4398	105.5
Wheatgrass + Cocksfoot + B. trefoil + Sainfoin +W. clover	12068	8563	4684	4483	2813	3249	5977	143.3
Average	11442	8568	4252	4081	2506	3183	5672	
LSD 5%	278	206	163	159	143	183	261	

Table 1. Dry matter yield by years

A similar trend was also found in the fourth year. The DM yields in mixtures with wheatgrass were lower than that of cocksfoot. In the last two years the DM yield from the pure wheatgrass was higher than that of pure cocksfoot (by 46% on average), due to the substantial reduction of cocksfoot. The mixtures of wheatgrass with perennial legumes were less productive.

The results averaged over the period showed that there were no differences in productivity between the mixtures of wheatgrass and those of cocksfoot. Wheatgrass was significantly less productive than cocksfoot in the first and second cut and more productive in the last one (Table 2). The average yield of wheatgrass was significantly lower than that of cocksfoot, by 10%. These results showed that growth intensity of wheatgrass was lower than that of cocksfoot in the early growing season (spring and summer), and more intensive in autumn. That confirmed the findings of Walton (1983).

In the mixtures of wheatgrass and cocksfoot with perennial legumes, the yields at the first and second cuts, as well as the average yields, were lower. It was due to the greater proportion of grasses and their competitiveness over the years (in the first one for cocksfoot and in last one for wheatgrass).

Swarde		II	III	IV	Average and	nual yield
Swarus	kg ha <sup>-1</sup>					%
Wheatgrass	1466	1264	910	531	4170	100.0
Cocksfoot	1757	1410	1004	402	4573	109.7
LSD 5%	185	103	65	48	296	
Birdsfoot trefoil	2674	1995	1656	421	6746	161.8
Sainfoin	3262	1869	1071	410	6611	158.5
White clover	2257	1756	1276	429	5719	137.1
Wheatgrass + Birdsfoot trefoil	2450	1904	1523	557	6434	
Wheatgrass + Sainfoin	2753	1913	1175	483	6324	
Wheatgrass + White clover	1438	1565	1140	615	4758	
Wheatgrass + B. trefoil + Sainfoin + W. clover	2563	1837	1461	527	6388	
Average	2301	1805	1325	546	5976	100.0
LSD 5%	241	138	116	68	283	
Cocksfoot + Birdsfoot trefoil	2730	1796	1356	597	6479	
Cocksfoot + Sainfoin	2907	1705	1340	538	6490	
Cocksfoot + White clover	1854	1695	970	477	4996	
Cocksfoot + B. trefoil + Sainfoin + W. clover	2697	1764	1282	613	6356	
Average	2547	1740	1237	556	6080	101.7
LSD 5%	198	116	94	61	246	
Wheatgrass + Cocksfoot + B. trefoil	2388	1843	1221	759	6210	
Wheatgrass + Cocksfoot + Sainfoin	2406	1822	1169	706	6103	
Wheatgrass + Cocksfoot + W. clover	1434	1350	1089	525	4398	
Wheatgrass + Cocksfoot + B. trefoil +	2200	1704	1204	690	5077	
Sainfoin+W. Clover	2380	1704	1204	089	3977	
Average	2152	1680	1171	669	5672	94.9
LSD 5%	189	123	116	84	261	

Table 2. Dry matter yield by cuts average for the period

#### Conclusions

Wheatgrass was less productive than cocksfoot at the beginning (first and second cut) and more productive at the end of vegetation (third and fourth cut). There were no significant differences in the productivity of wheatgrass and cocksfoot in terms of their companion legume species of birdsfoot trefoil, sainfoin and white clover.

## References

Katova A. (2007) Species and varieties of perennial grasses for high quality forage in Bulgaria. *Journal of Mountain Agriculture on the Balkans* 156–161.

Mannetje L.'t. (2006) Climate change and grassland through the ages - an overview. *Grassland Science in Europe* 11, 733–738.

Trnka M., Bartosova L., Schaumberger A., Ruget F., Eitzinger J., Formayer H., Seguim B. and Olesen J.E. (2011) Climate change and impact on European grasslands. *Grassland Science in Europe* 16, 39–51.

Walton P.D. (1983) *Production and Management of Cultivated Forages*. Reston Publishing Company, Reston, Virginia, 336 pp.

# Effect of endophyte infection in red fescue-red clover simple mixture

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# Abstract

Red fescue (*Festuca rubra*) is a perennial grass commonly infected by the fungal endophyte *Epichlöe festucae* in several different European habitats. In a greenhouse experiment, *F. rubra* plants with different endophyte status (E+= infected; E-= non-infected) were grown in monocultures and in simple mixture with red clover (*Trifolium pratense*). The purpose of the study was to determine the effect of endophyte infection on the competition of red fescue plants with red clover. Root and shoot growth of red clover was significantly depressed when grown in competition with E+ plants compared to E– plants. When grown in monoculture or in competition with red clover, root biomass of E+ red fescue plants was lower than that of E– plants, but no differences in shoot biomass between E+ and E– were found. No differences in red fescue plant biomass of infected plants and the reduction of red clover growth, that is, an allelopathic interaction.

Keywords: Epichloë, plant biomass, simple mixture, Festuca rubra, Trifolium pratense

## Introduction

Red fescue (*Festuca rubra*) is a perennial grass that is very persistent and tolerant to a wide range of ecological conditions. This species is commonly infected by the fungal endophyte *Epichlöe festucae* as it has been reported in grasslands from Spain (Zabalgogeazgoa *et al.*, 1999), Finland and Norway (Wali *et al.*, 2007). *Neotyphodium* and *Epichlöë* endophytes may increase herbivore resistance, and the tolerance to abiotic stresses of the host grass (Malinowski and Belesky, 2006). Most studies in this context have focused on monocultures of the host grass by comparing infected and non-infected plants.

In previous work, we reported that growth of several legume species (*Trifolium pratense*, *Trifolium subterraneum* and *Lotus corniculatus*) was lower in the presence of infected red fescue plants than when growing with non-infected plants (Vázquez de Aldana *et al.*, 2010). This could be due to a greater competitive ability of infected plants (e.g. greater plant biomass) or to allelopathic interactions, that is, the release of chemical compounds that alter the growth of companion plant species. Here, we determined the effect of endophyte infection on competition of red fescue with red clover (*Trifolium repens*). We wanted to know whether the *Epichloë* endophyte can increase red fescue growth in competition with red clover.

## Materials and methods

For this experiment we used one line of red fescue consisting of endophyte infected (E+) and endophyte free (E-) half-sib plants. This line was developed from a single red fescue plant originally infected by *Epichloë festucae* which was collected in the 'dehesa' grasslands of the province of Salamanca (western Spain) (Vazquez de Aldana *et al.*, 2010). Seeds obtained from E+ and E- plants were germinated and individual plants were grown in a glasshouse in pots containing a mixture of peat moss, perlite and sand. After four months, the presence
of *E. festucae* fungus was verified by isolation of the fungus from stem and leaf sheaths on potato and dextrose agar (Bacon and White, 1994). Two months later, plants of similar size were selected, each consisting of two tillers, and roots were trimmed to 5 cm, and individually transplanted into pots (15 cm diameter) containing the potting mix and placed in a glasshouse during eight weeks. The experiment consisted of monocultures of red fescue infected (FrE+) and non-infected (FrE–), monocultures of red clover (Tp), and mixtures of red fescue and red clover (FrE+/Tp and FrE–/Tp), with five replicates. Ten seeds of red clover were sown in each corresponding pot (in mixture with Fr or in monoculture). The experiment was conducted in a glasshouse (temperature 22/15 °C day/ night; ambient light conditions of late spring). Ten days after sowing the red clover, five seedlings were left in each pot. Plants were harvested four weeks after sowing of red clover. Shoot and root dry matter was determined for each species.

Data were analysed by means of ANOVA considering as factors competition (monoculture vs. mixture) and endophyte infection (E+ vs. E–) using SPSS statistics 19.

#### **Results and discussion**

When grown with red clover no significant differences in shoot or root dry matter were found with respect to the growth of red fescue in monoculture. Root growth of red fescue was affected by the presence of endophyte in the plant, but the effect on shoot growth was not statistically significant. In monoculture or in mixture, red fescue E+ plants had significantly lower (P < 0.0001) root dry matter than E– plants (Table 1). This suggests that E+ plants were more efficient in acquisition of below-ground resources, since with lower investment in root biomass, E+ plants had similar aboveground biomass than E– plants.

Regarding red clover growth, both shoot and root biomass productions were significantly depressed when growing in mixture with red fescue (Table 2). This decrease was significantly greater when growing in mixture with E+ (78% in shoot and 74% in root biomass) than with E- plants (39% in shoot and 26% in root biomass). Similar results have been found in endophyte-infected tall fescue (Malinowski *et al.*, 1999). The greater reduction of red clover growth when growing with E+ plants was not related to a greater root biomass or shoot biomass of E+ plants (in comparison with E-).

These results suggest that the greater competitive ability of E+ plants could be related to chemical interactions. Several compounds are known to have allelopathic activity and fungal endophytes can produce secondary metabolites in plants, or in greater concentrations than in E– plants, which may have an effect on the companion species. Although *Epichloë* and *Neotyphodium* endophytes only colonise aerial plant tissues, metabolism in roots could

	Dry matter (g plant <sup>-1</sup> )							
	Monoc	ulture	Mixture with red clover					
	Mean	SE	Mean	SE				
Shoot DM				÷				
E-	1.17	0.17	1.33	0.09				
E+	1.48	0.31	1.33	0.19				
Root DM				·				
E-	0.91	0.14	1.08	0.10				
E+	0.36	0.10	0.38	0.10				
Shoot: root				·				
E-	1.29	0.04	1.24	0.07				
E+	4.34	0.37	3.79	0.45				

Table 1. Endophyte effect on shoot and root dry matter (DM) of red fescue in response to competition with red clover

	Dry matter (mg plant <sup>-1</sup> )							
	Monoo	culture	Mixtu	Mixture with red fescue				
	Mean	SE	Endophyte status	Mean	SE			
Shoot DM	67.72	6.65	E-	44.11	9.64			
			E+	26.18	3.19			
Root DM	10.37	1.38	E–	5.45	1.37			
			E+	3.69	0.54			

Table 2. Shoot and root dry matter (DM) of red clover in response to competition with endophyte infected (E+) and non-infected (E-) red fescue (means of 5 replicates)

be affected by fungal infection. A greater concentration of total phenolic compounds was found in roots and exudates of tall fescue plants infected with *Neotyphodium* (Malinowski *et al.*, 1998) and in roots of red fescue infected with *Epichloë* (Vázquez de Aldana *et al.*, 2011).

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#### References

Bacon C.W. and White J.F. (1994) Stains, media, and procedures for analyzing endophytes. In: Bacon C.W. and White J.F. (eds) *Biotechnology of endophytic fungi of grasses*, CRC, Boca Raton, FL, pp. 47–56.

Malinowski D.P., Alloush G.A. and Belesky D.P. (1998) Evidence for chemical changes on the root surface of tall fescue in response to infection with the fungal endophyte *Neotyphodium coenophialum*. *Plant and Soil* 205, 1–12.

Malinowski D.P. and Belesky D.P. (2006) Ecological importance of *Neotyphodium* spp. grass endophytes in agroecosystems. *Grassland Science* 52, 1–14.

Malinowski D.P., Belesky D.P. and Fedders J.M. (1999) Endophyte infection may affect the competitive ability of tall fescue grown with red clover. *Journal of Agronomy and Crop Science* 183, 91–101.

Vázquez de Aldana B.R., Romo M., García-Ciudad A., Petisco C. and García-Criado B. (2010) Effects of fungal endophyte infection in the grass *Festuca rubra* on germination and growth of four legume species. *Grassland Science in Europe* 15, 368–370.

Vázquez de Aldana B.R., Romo M., García-Ciudad A., Petisco C. and García-Criado B. (2011) Infection with the fungal endophyte *Epichlöe festucae* may alter the allelopathic potential of red fescue. *Annals of Applied Biology* 159, 281–290.

Wali P.R., Ahlholm J.U., Helander M. and Saikkonen K. (2007) Occurrence and genetic structure of the systemic grass endophyte *Epichloe festucae* in fine fescue populations. *Microbial Ecology* 53, 20–29.

Zabalgogeazcoa I., Vazquez de Aldana B.R., Garcia Criado B. and Garcia Ciudad A. (1999) The infection of *Festuca rubra* by the fungal endophyte *Epichloë festucae* in Mediterranean permanent grasslands. *Grass and Forage Science* 54, 91–95.

# The impact of leaf dressing with Kristalon on the productivity of grass-legume mixtures in a 3-cut harvesting regime

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## Abstract

In this research we established that fertilizer methods considerably influenced the productivity of a legume-grass sward used for haymaking. Phosphoric and potash fertilizers ( $P_{90}K_{90}$ ) raised dry matter yield by 1.74 t ha<sup>-1</sup> in comparison with a variant without fertilization. Additional fractional treatment of nitrogen fertilizer ( $N_{90}$ ) (after 1 and 2 hay cuts) raised yield by 3.24 t ha<sup>-1</sup>. The total output of dry matter was thus increased to 9.78–11.28 t ha<sup>-1</sup>. Spraying of the vegetative mass by water-soluble fertilizer increased the yield in comparison with a variant without fertilizer by 0.79 t ha<sup>-1</sup>. Joint treatment of mineral fertilizers and a leaf-feeding dressing appeared to be more effective: the dry matter output increased by 2.68–4.19 t ha<sup>-1</sup> and thus was increased to 10.72–12.23 t ha<sup>-1</sup>. These variants also had the greatest quantity of vegetative shoots, 2025–2231 per m<sup>2</sup>, and percentage ratio of leaves, 59.5–62.2%; it also promoted an increase of the contents of a crude protein in hay dry matter, from 15.37 to 17.16%, and a decrease in crude fibre, from 26.55 to 25.85%.

Keywords: leaf-feeding dressing, legume-grass mixture, fertilization, productivity, hay quality

## Introduction

In the Forest-Steppe of Ukraine, legume-grass mixtures are the main source of high quality hay (Veklenko, 2007). But to obtain three full-value hay cuts per season grass fertilization is required. Except for the use of high-cost organic system of fertilization of haylands, and utilization of the cheapest renewable resources, first of all symbiotic nitrogen fixation by the legumes, the increase of mineral fertilizer efficacy is of great importance for hay producers. Therefore, in the same way that surface application of macro-elements in the form of traditional granulated mineral fertilizers, which have substantial influence on the rhizosphere of legume-grass agro-phytocenosis (Petrychenko *et al.*, 2007), water-soluble complex fertilizers with a balanced formulation of macro- and microelements on a chelates basis, which are non-toxic and completely assimilated by plants through the leaves, are of great interest. The aim of the research was to determine the efficacy of outside root nutrition of perennial haycrops, using the water-soluble complex fertilizer Kristalon<sup>TM</sup> and the possibility of its use against a background of the generally accepted mineral system of grass fertilization in Ukraine.

## Materials and methods

Research was carried out during 2007–2009 on the dark-grey podzol soil of the Forest-Steppe zone of Ukraine, the soil having average content of available phosphorus, exchangeable potassium and low nitrogen content. The legume-grass mixture consisted of timothy (*Phleum pratense* L.), reed fescue (*Festuca orientalis* (Hack.) V. Krecz. et Bobr.), red clover (*Trifolium pratense* L.) and birds-foot trefoil (*Lotus corniculatus* L.p.p.). Variants of mineral fertilizer used were as follows: 1 – without fertilizer (control);  $2 - P_{90}K_{90}$ ;  $3 - N_{90}P_{90}K_{90}$ ; 4

– Kristalon<sup>TM</sup>;  $5 - P_{90}K_{90}$  + Kristalon<sup>TM</sup>;  $6 - N_{90}P_{90}K_{90}$  + Kristalon<sup>TM</sup>.  $P_{90}K_{90}$  was applied to soil once per season in autumn.  $N_{90}$  was applied separately in amounts of  $N_{30}$  for each haycrop. Vegetative mass of the grass stand was sprayed by a solution in water of Kristalon<sup>TM</sup> three times a season at the phase of grass shooting and branch formation of the legumes. The rate of its use was 4 kg ha<sup>-1</sup> in – 250 l ha<sup>-1</sup> of water application. Kristalon<sup>TM</sup> contains: N – 18%, P – 18%, K – 18%, Mg – 3%, S – 2%, B – 0.025%, Cu – 0.01%, Mn – 0.04%, Fe – 0.07%, Mo – 0.004%, Zn – 0.025%. The regime of grass-stand use is triple mowing. The size of sowing plots is 25 m<sup>2</sup>, their location on the area is systematic in two layers, with four replications. Grass-stand density, plant height, botanical composition, foliation and chemical composition were determined before each yield measurement. Together with recording of green mass yield (YM), the output of dry mass (DM), crude protein (CP) and gross energy (GE) per ha was determined.

#### **Results and discussion**

In the control without fertilization, the grass stand had the lowest productivity, sod density and plant height (Table 1). Application of  $P_{90}K_{90}$  increased plant density by 19% and raised content of legume components due to reduction of grasses. Phosphorus-potassium fertilization helped to get an additional 9.78 Mg ha<sup>-1</sup> YM, 1.74 Mg ha<sup>-1</sup> DM, 0.34 Mg ha<sup>-1</sup> (CP) and 30.2 GJ ha<sup>-1</sup> (GE) in comparison with the unfertilized control.

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	Density	,	Yield	Leaves	YM	DM	CP,	GE
Treatments	stems	Height, m	proportion,	content,	M = 1 ==	Ma ha-l	Mg	CLbl
	per m <sup>2</sup>	_	g kg <sup>-1</sup>	%	Mg na	Mg na ·	ha <sup>-1</sup>	GJ na ·
Control – without	1570	0 49*/0 26*:	*725*/007**	560	24 61	8.04	1 00	140.6
fertilizer	1372	0.48*/0.50**	1554/22144	30.2	54.01	8.04	1.08	140.0
$P_{00}K_{00}$	1865	0.51/0.38	699/269	58.5	44.39	9.78	1.42	170.8
$N_{90} P_{90} K_{90}$	2045	0.55/0.40	802/184	61.0	52.62	11.28	1.81	199.2
Kristalon	1784	0.50/0.37	718/254	57.4	39.89	8.83	1.24	153.7
P <sub>90</sub> K <sub>90</sub> + Kristalon	2025	0.52/0.39	699/277	59.5	47.90	10.72	1.62	188.3
N <sub>90</sub> P <sub>90</sub> K <sub>90</sub> + Kristalon	2231	0.57/0.41	803/191	62.2	56.65	12.23	2.08	216.3
LSD <sub>0.05</sub>	63	0.05/0.08	3.0/2.0	0.09	0.53	0.13	0.02	2.39

Table 1. Productivity of legume-grass mixture depending on fertilization (average for 2007-2009)

\* - in numerator - grasses, \*\* - in a denominator - legumes.

Application of mineral nitrogen ( $N_{90}$ ) in legume-grass stand, together with phosphoruspotassium fertilizer, increased quantity of grass in the yield from 735 to 802 g kg<sup>-1</sup> and, correspondingly, reduced quantity of legumes from 227 to 184 g kg<sup>-1</sup>. This occurred against



Figure 1. Average content and standard deviation of crude protein and crude fibre in dry matter of hay depending on the variants of fertilization (average for 2007–2009)

a background of the increase of the general sod density in comparison with the control up to 2045 sprouts per m<sup>2</sup>.

Substantial increase of grass plant height was recorded; a 50% increase of YM, 40% rise of DM and GE and 67% increase of CP output from the cropping area. Spraying of vegetative mass of grasses by Kristalon<sup>TM</sup> (variant 4) was less efficient than the variant with fertilizer  $P_{00}K_{00}$  but their combination was almost equal to the variant with complete mineral fertilizer  $N_{00}P_{00}K_{00}$ . The best indices of hayland productivity formation were obtained under joint application of phosphorus-potassium fertilizer (P<sub>00</sub>K<sub>00</sub>), mineral nitrogen fertilizer  $(N_{30+30+30})$  and outside root nutrition by Kristalon<sup>TM</sup> (variant 6). In response to this variant of fertilization, the grass density also increased to 2231 sprouts per m<sup>2</sup> and the share of leaves in the mass of a plant was increased to 62.2% facilitating the increase of raw material quality. Figure 1 shows CP and crude fibre (CF) on average for the years of the research, in examples of the grass by the variants of the research. Control variant without fertilization had the lowest level of the average content of CP  $(13.73 \pm 1.80\%)$ , while in contrast the CF content was higher  $(27.51\pm0.50\%)$ . Variants of the joint application of Kristalon<sup>TM</sup> with  $P_{q_0}K_{q_0}$  and especially with  $N_{q_0}P_{q_0}K_{q_0}$  increased CP content in dry mass of hay up to 15.37–17.16% and caused the reduction of CF in it up to 26.55–25.87%. Regression analysis carried out by us showed positive correlation between foliation of the grass stand and crude protein content in the dry mass of hay. In addition, as shown in Figure 2, such dependence was revealed in each of three hay cuttings per season, as indicated by the determined coefficients of correlation r = 0.5286 - 0.6149 (P = 0.0241 - 0.0070).



Figure 2. Correlative relations between leaf content and crude protein content in dry matter of hay by the hay cuttings (average for 2007–2009)

#### Conclusions

Research has shown the expediency of outside root nutrition of a legume-grass stand of haymaking use. The most effective method of fertilization of a mixture of timothy, reed fescue, red clover and birds-foot trefoil under a 3-cut mowing regime vegetation period was with complex supply with macro- and microelements combining the surface application of starting doses of mineral nitrogen ( $N_{30}$ ) with outside root nutrition by Kristalon<sup>TM</sup> (4 kg ha<sup>-1</sup>) under each hay-crop, against a background of 90 kg ha<sup>-1</sup> of phosphorus-potassium fertilizers applied as a reserve before the beginning of grass vegetation.

#### References

Petrychenko V.F., Kovtun K.P. and Veklenko Y.A. (2007) Agroecological aspects of nitrogen fixation intensification for the productivity increase of meadow grass stands on the arable lands of the Forest-Steppe of Ukraine. *Grassland Ecology* 7, 234–342.

Veklenko Y.A. (2007) Productivity formation of cultural pastures' agrophytocenosis in the Forest-Steppe of Ukraine depending on the techniques of their creation and practices of use. *Grassland Ecology* 7, 224–230.

## Session 1.2.

**Future of pastoral/grazing systems** 

## **Innovations in grazing**

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## Abstract

Current trends in livestock farming in Europe are causing a decline in the popularity of grazing. Since, in general, society favours grazing and in most situations it is economically attractive, support for grazing is useful. This paper discusses recent innovations to support grazing. Support is especially needed for those situations where grazing is often under debate, e.g. on farms with automated milking systems and/or large herds. Four categories of innovations are discussed. First, technical support, such as automatic sward-height measurements, GPS or mobile automated milking systems. Second, novelties in grazing systems. Since increased herd size makes grazing management more difficult, relatively simple grazing systems have been developed. Third, decision support tools for farmers on a day-to-day basis. Fourth, projects to stimulate grazing. These projects should focus preferably not only on knowledge transfer, but also on the needs and personal preferences of the farmer.

Keywords: innovations, grazing

## Introduction

The general public in Western European countries is increasingly calling for farm systems in which animals can display their natural behaviour, and grazing is an important aspect of such behaviour. In addition to animal welfare, an open landscape with grazing cattle is highly appreciated. Also due to grazing, the biodiversity of the landscape increases which is valued by society. However, current trends in livestock farming in Europe cause a decline in the popularity of grazing (Van den Pol-van Dasselaar *et al.*, 2008). Support for grazing is useful from a societal and economic perspective. Support is especially needed for those situations where grazing is often under debate, e.g. on farms with automated milking systems and/or large herds. Van den Pol-van Dasselaar *et al.* (2010) showed that even in these situations grazing is economically attractive. However, grazing is then more complicated and farmers do not employ grazing. This paper provides an insight into the magnitude of grazing in Europe and discusses recent innovations to support grazing. The topic will be further discussed and elaborated during a workshop 'Innovations in grazing' of the EGF Working Group 'Grazing' during the 24<sup>th</sup> EGF meeting in Poland in 2012.

## Materials and methods

Data on grazing in Europe are not easily available. Even though some countries have some statistics available, the majority of countries have no reliable statistical data on grazing. To obtain an insight into grazing, a survey was conducted among members of the EGF Working Group 'Grazing' in October and November 2011. The members were asked to provide an educated guess on the amount of grazing dairy cattle in their country and to report on recent innovations. Data on grazing were compared with results from earlier surveys among members of the EGF Working Group 'Grazing' (Van den Polvan Dasselaar *et al.*, 2011).

## **Results and discussion**

The percentage of dairy cattle grazing varies between the different countries. Even though the data are often only an educated guess, it is clear that in general, the popularity of grazing is declining:

- Norway, Sweden, Finland: welfare legislation, six weeks to four months outside, results of 2011 show that the number of hours that cattle spent outside is decreasing.
- Denmark: 84% in 2001, 70% in 2003, 40–50% in 2008, 35–45% in 2010, 30–35% in 2011.
- Ireland: 99% in 2010 and 2011, staying consistently high, grass-based seasonal systems dominate.
- the Netherlands: 90% in 2001, 85% in 2004, 80% in 2007, 74% in 2010, 70–75% in 2011.
- Belgium: 85–95% in 2010 and 2011.
- Luxembourg: 90% in 2008, 75-85% free access in 2010, but 10% real grazing.
- Germany: along the Alps and low mountain range 85% in 2010, other regions grazing is marginal, decreasing, 30% in 2011 in northern Germany.
- France: decreasing in the more intensive area, but not in wet mountain conditions, 90–95% in 2011.
- Switzerland: 85–100% in 2011.
- Austria: 25% in 2011.
- Poland: decreasing.
- Estonia: 35% in 2011.
- Czech Republic: 20% in 2010, sharp decrease in 1990-2008, currently slight increase.
- Bosnia and Herzegovina: 5% in 2011.
- Slovenia: 25% in 2010, stable or decreasing.
- Portugal: 50% in 2010, increasing.
- Spain: 20% in 2010 in NW, rest 0%, slow increase.
- Greece: 15% in 2010, less than 10% in 2011.

With respect to innovations in grazing, we identified four categories of innovations. First, technical support (hardware). New developments include mobile automated milking systems and grass yield measurements. Mobile automated milking systems are currently available in Denmark, France and Belgium. Even though this milking technology may not be adopted in practice by the majority of farmers, it will be useful in particular situations, e.g. in remote areas which are not easily accessible for grazing. This technology requires further operational refinement, e.g. buffer feeding during the transition periods and the transport of cows has to be improved. Also, there is an urgency for the development of methods to determine yield and quality in a simple and quick way (rapid pasture meter, remote sensing). First results of automatic sward-height measurements in Demark using equipment developed in New Zealand are promising (Oudshoorn et al., 2010). Other innovations are automatic fence gates in the fields and automatic measurement of grass intake by sensor technology. A second group of innovations are novelties in grazing systems. During the last few years, however, there have been no major developments. The most often mentioned relatively new system is the rediscovery of continuous grazing instead of rotational grazing. This grazing system is also advantageous for large herds since it is relatively easy to manage. In some cases, systems such as simplified rotational grazing systems, flexible part-time grazing to buffer variations in pasture quantity and quality, and night time grazing are mentioned. Night time grazing ensures that animals are outside for a large part of the day, but the actual grass intake is relatively low (Chapinal et al., 2010).

A third group of innovations are decision support tools for farmers. In many countries these are currently developed or there is a wish to develop them. Especially in Ireland, many tools and models are available for farmers and Irish farmers actually use them. The tools which have been developed in recent years are innovative tools for farmers to maximise profitability, such as profit monitors and grazed pasture feed-budgeting tools. Also in other countries, decision support tools for farmers are available on a day-to-day basis, e.g. GrazeVision in the Netherlands (Zom and Holshof, 2011). Some countries, e.g. Austria and France, have developed grazing information platforms, where all the available information is easily accessible for farmers. Even though there has been a great deal of development in decision support tools are needed that are automatically populated with data, e.g. grass yield, grass intake, climate and weather, and which provide support for grassland management decisions.

A fourth category of innovations in grazing are projects to stimulate grazing. This category is needed to make the innovations from the previous three categories work. Of course, workshops, seminars, publications and courses for professional training of farmers and advisors on grazing strategies are necessary. Experiences in the Netherlands (www.koeenwij. nl) showed, however, that the most successful projects focus not only on knowledge transfer, but also on the needs and personal preferences of the farmer (Van den Pol-van Dasselaar *et al.*, 2008).

People from many countries indicate that there is an urgent need to develop technical support for grazing, grazing systems and decision support tools for grazing. The decision support tools should be capable of adaptation to different conditions and management systems. Furthermore, exchange of knowledge is highly appreciated since the possibilities for grazing research are limited. Therefore, an international workshop 'Innovations in grazing' will be held prior to the 24<sup>th</sup> General Meeting of the European Grassland Federation in Poland in 2012. In the workshop, recent innovations in grazing will be elaborated.

#### Conclusions

The popularity of grazing in Europe is declining. Since this is an undesirable trend from an economic and societal point of view, innovations to support grazing are needed. Furthermore, it is essential to exchange knowledge about the already available innovations in several regions of Europe. The EGF Working Group 'Grazing' is a valuable platform for this.

#### References

Chapinal N., Goldhawk C., de Passillé A.M., von Keyserlingk M.A.G., Weary D.M. and Rushen J. (2010) Overnight access to pasture does not reduce milk production or feed intake in dairy cattle. *Livestock Science* 129, 104–110.

Oudshoorn F.W., Hansson S.L. and Hansen H. (2010) Calibration of the C-Dax Pasture Meter in a Danish Grazing system. *Grassland Science in Europe* 15, 166–168.

Van den Pol-van Dasselaar A., Vellinga T.V., Johansen A. and Kennedy E. (2008) To graze or not to graze, that's the question. *Grassland Science in Europe* 13, 706–716.

Van den Pol-van Dasselaar A., de Haan M., Evers A. and Philipsen A.P. (2010) Simulation of the effect of grass intake on the farmer's income. *Grassland Science in Europe* 15, 100–102.

Van den Pol-van Dasselaar A., Hennessy D., Peyraud J.L., Pinxterhuis J.B. and de Vliegher A. (2011) *Research methodology of grazing*. Proceedings EGF Working Group Grazing. Report 405. Wageningen UR Livestock Research.

Zom R.L.G. and Holshof G. (2011) GrazeVision: A versatile grazing decision model. *Grassland Science in Europe* 16, 226–228.

## Effects of grazing severity on early lactation dairy cow performance

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## Abstract

A grazing experiment was undertaken to investigate the effect of post-grazing sward height (PGSH) on early lactation dairy cow performance. Ninety Holstein-Friesian dairy cows (mean calving date February 13±17.7 days) were randomly assigned across three PGSH treatments: 2.7 cm (very severe, VS), 3.5 cm (severe, S) and 4.2 cm (moderate, M) from February 14 to April 24 2011. Increasing PGSH from 2.7 to 3.5 to 4.2 cm linearly increased (P < 0.001) milk yield (22.5, 23.6 and 25.1 kg cow<sup>-1</sup>day<sup>-1</sup>, respectively) and milk solids yield (1.75, 1.91 and 2.00 kg cow<sup>-1</sup>day<sup>-1</sup>, respectively). Grazing to 2.7 cm reduced (P < 0.001) cumulative milk yield ( $-160 \text{ kg cow}^{-1}$ ), cumulative milk solids yield (-17 kg milk/cow), milk fat content ( $-2.5 \text{ g kg}^{-1}$ ; P < 0.05), milk protein content ( $-1.0 \text{ g kg}^{-1}$ ; P < 0.05), end body condition score (-0.13; P < 0.01) and induced higher body condition score loss (-0.15; P < 0.05) when compared to the S and M treatments which performed similarly (1538 kg cow<sup>-1</sup>, 124 kg cow<sup>-1</sup>, 46.4 g kg<sup>-1</sup>, 34.1 g kg<sup>-1</sup>, 2.84 and -0.21, respectively). The results of this study indicate the limited benefit of grazing swards to 4.2 cm in early spring and the reduced animal performance when grazing to 2.7 cm. Grazing to 3.5 cm achieved the balance between sward utilization and cow productivity.

Keywords: dairy cows, early lactation, post-grazing height, utilization

## Introduction

Grass when grazed efficiently is the cheapest feed available on the dairy farm (Finneran *et al.*, 2010). With the abolition of milk quotas in 2015, dairy enterprises in Ireland must seize the opportunity to expand their grass-based production systems and secure their competitive position on the European market. This can be achieved through early spring turnout of the post-parturient cow which will maximize the use of grazed grass in the dairy cow's diet. The availability of herbage, however, is limited in early spring. Grazing pastures to lower post-grazing sward heights (PGSH) than that currently recommended (4.0 cm) during the first grazing rotation would ensure greater herbage availability during this period. A number of studies exploring stocking rate (SR) systems reported that although high SR systems achieve high levels of utilization by grazing to a low PGSH, a decline in milk production per cow is classically identified (Michell and Fulkerson, 1987; MacDonald *et al.*, 2008). In these studies, PGSH was intertwined with SR. Therefore, the current study investigated the true effect of PGSH on early lactation animal performance.

## Materials and methods

Ninety (27 primiparous and 63 multiparous) spring calving Holstein Friesian dairy cows were balanced on calving date (13 February; s.d. 17.7 days), lactation number (2.1; s.d. 1.05), previous lactation (first 37 weeks) milk yield (4591; s.d. 682.7 kg), mean bodyweight

(BW; 482, s.d. 57.8 kg) and mean body condition score (BCS; 2.92, s.d. 0.141). The experiment was a randomised block design. Animals were randomly assigned pre-calving across three PGSH treatments (n = 30): 2.7 cm (very severe – VS), 3.5 cm (severe – S) or 4.2 cm (moderate – M) from February 14 to April 24. An equal area was assigned to each treatment and treatments were managed independently, with an overall stocking rate of 2.73 cows ha<sup>-1</sup>. Fresh herbage was allocated daily. Herbage mass (HM) was calculated twice weekly by cutting two strips per grazing treatment. Pre and post-grazing heights were measured daily. The difference in PGSH was achieved by ensuring a 2.5 kg DM cow<sup>-1</sup> day<sup>-1</sup> difference in daily herbage allowance (DHA) between treatments. All animals were supplemented with 3.4 kg DM of concentrate/day throughout the experiment. Grass dry matter intake (GDMI) was measured using the *n*-alkane technique (Dillon and Stakelum, 1989) during week 5 (March 12-18) and week 9 (April 11-16) of the experiment. Pasture utilization was calculated for each grazing rotation from the pre-grazing yield relative to the post-grazing yield. Milk yield was recorded daily; milk composition, BW and BCS were measured weekly. Animal variables were analysed using covariate analysis and the PROC MIXED statement of SAS with terms for parity, treatment, the interaction of treatment and parity. Days in milk and the pre-experimental values were used as covariates.

#### **Results and discussion**

The M treatment had higher pre-grazing HM (+120 kg DM ha<sup>-1</sup>; P < 0.01) and pre-grazing sward height (+0.60 cm; P < 0.001) when compared to the VS and S treatments that recorded similar values (956 kg DM ha<sup>-1</sup> and 6.40 cm, respectively). Mean DHA (> 2.7 cm) were 7.7, 10.0 and 12.1 kg DM cow<sup>-1</sup> and mean PGSH were 2.7, 3.5 and 4.2 cm for the VS, S and M treatments, respectively. Results from the first GDMI measurement period show that GDMI was similar between the S and M treatment animals (10.8 kg DM cow<sup>-1</sup>) but it was 3.1 kg DM less for the VS animals (7.7 kg DM cow<sup>-1</sup>; P < 0.05), all animals were offered 4.0 kg DM concentrate per day. The increase in PGSH from 2.7 to 3.5 to 4.2 cm during the second measurement period linearly increased (P < 0.001) GDMI: 13.7, 15.9 and 16.8 kg DM cow<sup>-1</sup>, respectively. Throughout the experiment, the increase in GDMI was associated with an increase (P < 0.001) in milk yield from 22.5, 23.6 and 25.1 kg cow<sup>-1</sup> day<sup>-1</sup> for the VS, S and M animals, respectively. Increasing PGSH from 2.7 to 3.5 to 4.2 cm resulted in a linear increase in protein yield (750, 807 and 857 g day<sup>-1</sup>, respectively). Fat yield was lower for the VS animals (-125 g day<sup>-1</sup>; P < 0.001) when compared to the S and M animals (1126 g day<sup>-1</sup>). Milk solids yield increased (P < 0.001) by +0.16 and +0.09 g day<sup>-1</sup> from VS

	Post-grazing	sward heig	Level of significance		
	VS	S	M	S.E.D. <sup>1</sup>	<i>P</i> -value
Milk yield, kg cow <sup>-1</sup>	22.5ª	23.6 <sup>b</sup>	25.1°	0.51	0.001
Milk fat content, g kg <sup>-1</sup>	43.9ª	46.8 <sup>b</sup>	45.9 <sup>b</sup>	0.91	0.015
Milk protein content, g kg <sup>-1</sup>	33.1ª	34.1 <sup>b</sup>	34.0 <sup>b</sup>	0.35	0.022
Milk lactose content, g kg <sup>-1</sup>	46.8	46.9	47.0	0.22	0.636
Milk solids yield, kg day <sup>-1</sup>	1.75 <sup>a</sup>	1.91 <sup>b</sup>	2.00°	0.46	0.001
End body weight, kg	442ª	451 <sup>ab</sup>	464 <sup>b</sup>	7.3	0.019
Body weight change, kg day <sup>-1</sup>	-0.92	-0.66	-0.66	0.212	0.319
End body condition score	2.71ª	2.80 <sup>b</sup>	2.87 <sup>b</sup>	0.041	0.004
Body condition score change	-0.36ª	-0.23 <sup>b</sup>	-0.18 <sup>b</sup>	0.051	0.005

Table 1. Effect of post-grazing sward height (VS = 2.7 cm, S = 3.5 cm and M = 4.2 cm) on animal performance during the early lactation period (February 14 – April 24)

<sup>1</sup> SE of the difference.

<sup>a-c</sup> Means within a row with different superscripts differ (P < 0.05).

to S and from S to M treatment groups, respectively (Table 1). Very severe grazing depressed (P < 0.01) cumulative milk yield ( $-160 \text{ kg cow}^{-1}$ ) and cumulative milk solids yield (-17 kg milk cow<sup>-1</sup>), when compared to the S and M treatments which performed similarly (1538 kg milk per cow and 124 kg milk solids cow<sup>-1</sup>). The severe decrease in production reflected the high level of restriction placed upon the VS treatment animals as they were offered a very low DHA to achieve the desired PGSH. Grass utilization (> 2.7 cm) was, however, maximized (P < 0.001) by grazing to 2.7 cm (0.94) when compared to 3.5 cm (0.82) and 4.2 cm (0.74). This was associated with an increase in actual grazed area from 14.9 ha (VS) to 16.1 ha (S) to 19.0 ha (M) at the end of the first grazing rotation (March 31).

#### Conclusions

Results from the current experiment show that grazing to 2.7 cm physically restricts cows from grazing further into the sward thereby lowering GDMI which consequently results in milk and milk solids production losses. The low level of grass utilisation on the M treatment and the similar cumulative milk and milk solids production between the S and M animals clearly demonstrate the limited benefit of grazing swards to 4.2 cm in early spring. It is also clear that grazing to 2.7 cm is too restrictive and should be avoided. This study concludes that perennial ryegrass swards grazed in early spring should be grazed to 3.5 cm to achieve a balance between pasture utilisation and animal production performance.

## References

Dillon P. and Stakelum G. (1989) Herbage and dosed alkanes as a grass management technique for dairy cows. *Irish Journal of Agricultural Research* 8, 104 (Abstract).

Finnernan E., Crosson P., Shalloo L., Foristal D., O'Kiely P. and Wallace M. (2010) Simulation modelling of the cost of producing and utilising feeds for ruminants on irish farms. *Journal of Farm Management* 14, 95–116.

MacDonald K.A., Penno J.W., Lancaster J.A.S. and Roche J.R. (2008) Effect of stocking rate on pasture production, milk production, and reproduction of dairy cows in pasture-based systems. *Journal of Dairy Science* 91, 2151–2163.

Michell P. and Fulkerson W.J. (1987) Effect of grazing intensity in spring on pasture growth, composition and digestibility, and on milk production by dairy cows. *Australian Journal of Experimental Agriculture* 27, 35–40.

## Effects of breed on foraging sites and diet in dairy cows on mountain pasture

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#### Abstract

Biodiverse semi-natural pastures are threatened because of sub-optimal grazing. The influence of breed on choice of foraging vegetation type, diet and hence pasture management was investigated in dairy cows kept on mountain pastures. Five dairy cows each of the traditional Swedish Mountain cattle breed and the commercial Holstein breed were equipped with GPS receivers measuring animal position for 6 h daily grazing time during 6 days. Plant groups in the ingested herbage were recorded visually for 30 min per cow and day. The grazing area, mapped using infra-red aerial photography combined with field work, consisted of ten vegetation types dominated by bilberry forest (33%), mixed forest (28%) and grass and sedge fen (12%). Although grass-dominated pasture comprised only 0.3% of the area, the cows spent on average 27% of their time there. Swedish Mountain cows spent less time in grass-dominated pasture than Holsteins (24% vs. 31%) but more time in bilberry forest (21 vs. 13%). Swedish Mountain cattle also travelled longer distances during grazing (6.3 vs. 5.0 km). This limited study revealed a general selection of grass-dominated pasture, but indicated that using traditional breeds can result in better management of other vegetation types.

Keywords: cattle, grazing, vegetation, selection

## Introduction

The semi-natural grasslands of northern Europe are habitats with a wide diversity of valuable plant and animal species. They are under threat because of the cessation of grazing in modern farming. In this context, multifunctional agriculture using summer farming areas in the traditional way can play an important role. Historically, village livestock spent their summers on mountain pasture, which provided an important complement to the feed supply at home. Animal breed selection has often been suggested as a tool for obtaining specific grazing effects, as livestock bred in traditional nutrient-poor environments may differ from livestock selected in more fertile environments (Rook *et al.*, 2004). For mountain pastures, differences in vegetation and diet selection between low-yielding traditional and high-yielding modern dairy cows have previously been reported by Sæther *et al.* (2006). However, to our knowledge, breed comparison studies of cows with similar milk yield are sparse. The aim of this study was to determine the foraging behaviour and site selection of two contrasting dairy cow breeds grazing mountain pastures and to identify selected foraging areas, thus contributing to the improvement of grazing management strategies.

#### Materials and methods

The experiment was conducted over six days in late summer 2009 on mountain pasture at a summer farm in central Sweden (62°21'22" N; 13°20'19" E; elevation 810 m a.s.l.). The

heterogeneous pasture consisted of ten vegetation types, which were mapped by area using infra-red aerial photographs combined with field work.

Cows were released onto the pasture in the morning and returned voluntarily after, on average, 6 h. On their return, the cows were kept indoors until the next morning and were fed water, grass hay and concentrate. For the experiment, five cows of the traditional Swedish Mountain cattle breed (mean body weight 370 kg) and five cows of the modern Holstein breed (mean body weight 550 kg) within the herd were used. On average across breeds, their daily milk yield was 12.4 kg. Manual observations of foraging and ingested plant groups (grasses, herbs, sedges/rushes, dwarf shrubs, bushes/trees, fungi) were performed by focal sampling during 30 min per cow and day. Animal positions were recorded with GPS receivers (GPS Plus 2, Vectronic Aerospace GmbH, Berlin, Germany). Data of animal position, behaviour and pasture were merged in ArcMap version 9.2 (ESRI, Redlands, USA).

Selection ratio for vegetation type was defined as:

Time spent in the vegetation type as a proportion of total time on pasture Area of the vegetation type as a proportion of the total grazing area

Breed effects were analysed in an ANOVA with breed, individual and day as fixed factors in the GLM procedure (SAS Institute Inc. Release 9.2. Cary, USA). Data on time spent in the vegetation types and on plants ingested were arcsin-transformed before analysing. Selection ratios among the vegetation types were compared in Friedman's test, FREQ procedure, SAS.

## Results

Although the single patch of grass-dominated pasture only comprised 0.3% of the area, the cows of both breeds spent on average 27% of their time there, resulting in a much higher selection ratio for this vegetation type than the others (P < 0.002). Mixed forest was also selected (P < 0.002), but all other vegetation types had selection ratios below 1.0 and hence were avoided (Figure 1). The Swedish Mountain cows travelled longer distances than the Holstein cows (Table 1) and spent less time in the grass-dominated pasture (24% vs. 31%; P = 0.007), but more time in bilberry forest (21 vs. 13%; P = 0.006).

Table 1. Time spent on pasture, distance travelled, proportion of time spent foraging, and proportion of time spent foraging grasses, herbs, sedges/rushes, dwarf shrubs, bushes/trees and fungi per day by Swedish Mountain (n = 5) and Holstein (n = 5) cows on mountain pasture; SEM = standard error of the mean

Parameter	Swedish Mountain	Holstein	SEM	Р
Pasture time, h	6.1	6.4	0.2	0.073
Distance, km	6.3	5.0	0.2	< 0.001
Foraging, %	78.9	85.2	3.3	0.161
Grass, %	79.3	84.5	4.9	0.516
Herbs,%	0.2	0.3	0.2	0.994
Sedges/rushes, %	17.0	9.9	4.8	0.345
Dwarf shrubs, % <sup>a</sup>	2.3	4.0	1.2	-
Bushes/trees, % <sup>a</sup>	0.4	1.3	0.3	_
Fungi, %ª	0.8	0.0	0.2	_

<sup>a</sup>Too low proportions for differences to be analysed.



Figure 1. Selection ratio of five Swedish Mountain and five Holstein dairy cows for vegetation types on mountain pasture consisting of grass-dominated pasture (GP, 0.3%); grass and sedge fen (GF, 11.8%); wooded grass and sedge fen (SF, 7.7%); wet fen (WF, 4.4%); felling patch (FP, 3.0%); bilberry forest (BF, 33.1%); mixed forest (MF, 27.7%); lichen-rich Scots pine forest (LF, 4.8%); lichen heath (LH, 2.0%); and forest lake (FL, 5.3%)

#### Discussion

The results from this limited study indicate that cows of a modern breed selected foraging areas with more digestible feeds, such as grass, to a higher extent than cows of a traditional breed, although no differences in proportion of grass ingested were found. The traditional breed travelled longer distances during grazing than the modern breed, which may be explained by more dispersed feed in the bilberry forest than in the grass-dominated pasture. The results are in agreement with previous studies (Sæther et al., 2006; Dumont et al., 2007). Contrary to Sæther et al. (2006), the differences in selection of vegetation type and diet observed here cannot be explained by differences in milk yield, but by a possible breed effect, caused for example by differences in body weight (Rook et al., 2004). Cows of both breeds clearly selected to graze the patch of grass-dominated pasture. In general, the cows, after roaming around by themselves or in small groups, ended up together on the grass-dominated pasture, which was situated at a neighbouring summer farm. The pasture had historically been mowed and grazed, which had resulted in a dense grass-dominated flora with herbs and some sedges. The combination of high plant density and high nutrient content on the frequently managed patch is a probable explanation for its popularity and is in accordance with previous studies (Dumont *et al.*, 2007). The short distance to the home farm, 500 m, where no such grassland was available, could also have contributed to the selection of the patch. The cows to some extent also selected mixed forest for grazing. This vegetation type contained plenty of wavy hair-grass (Deschampsia flexuosa), which may explain this choice.

#### Conclusions

This limited study revealed a general selection of grass-dominated areas in dairy cows on mountain pastures, but indicated that there is a better chance of also getting other vegetation types grazed when using a traditional breed, for instance Swedish Mountain cattle, rather than modern Holsteins.

#### References

Dumont B., Rook A.J., Coran C. and Rover K-U. (2007) Effects of livestock breed and grazing intensity on biodiversity and production in grazing systems. 2. Diet selection. *Grass and Forage Science* 62, 159–171. Rook A.J., Dumont B., Isselstein J., Osoro K., Wallis de Vries M.F., Parente G. and Mills J. (2004) Matching type of livestock to desired biodiversity outcomes in pastures – a review. *Biological Conservation* 119, 137–150.

Sæther N.H., Sickel H., Norderhaug A., Sickel M. and Vangen O. (2006) Plant and vegetation preferences for one old native and one modern Norwegian dairy cattle breed when grazing on shared semi-natural mountain pasture. *Animal Research* 55, 367–387.

# Effect of different grazing systems on sward structure during the first vegetation season after management introduction

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## Abstract

The aim of this work was to study the effect of different grazing management on sward structure in an upland grassland, during the first vegetation season after management introduction. The following treatments were applied in randomized blocks: intensive continuous stocking (C), extensive rotational grazing with two grazing cycles (2R), intensive rotational grazing with four grazing cycles (4R) and unmanaged control (U). The samples of biomass were collected by cutting (0.1 m × 0.1 m) monthly during the whole vegetation season from May to December. The functional groups of tall and short grasses had the fastest responses to different management and the significant differences were apparent in June. The accumulation of dead material between treatments differentiated following the August sampling and reflected grazing intensity C < 4R < 2R < U. The dominant short grass *A. capillaris* had a greater proportion of biomass in U and 2R treatments, significantly affected the sward structure of upland grassland during the first vegetation season after management introduction. The key factor affecting sward structure during the vegetation season is the behaviour of dominant species, plant biomass growth and dead material accumulation.

Keywords: continuous stocking, rotational grazing, functional groups, plant above-ground biomass

## Introduction

Numerous studies performed all over the world have confirmed a positive effect of grazing on the structure and composition of grasslands (e.g. WallisDeVries *et al.*, 1998). Vegetation changes in grazed grasslands occur due to a diverse mechanism of response of individual species to different grazing regimes and season (Bullock *et al.*, 2001). However, studies that have evaluated changes during the whole vegetation season are rare. Therefore, the objective of this investigation was to evaluate the effect of different grazing systems on sward structure during the course of the first vegetation season after management introduction.

## Materials and methods

The experiment was established over a five-year period of intensively grazed plots with *Agrostis capillaris* grassland dominancy (Oldrichov Grazing Experiment; Pavlů *et al.*, 2007) in 2002. The study site is situated in the Jizera Mountains, 10 km north of the city Liberec, Czech Republic. The average total annual precipitation is 803 mm, the mean annual temperature is  $7.2^{\circ}$ C (Liberec meteorological station) and the altitude is 420 m a.s.l Treatments were performed by controlled manipulation with exclosure cages (1 m × 1 m) as

follows: intensive continuous stocking (C) without using exclosure cages, intensive rotational grazing with four grazing cycles (4R), extensive rotational grazing with two grazing cycles (4R) and unmanaged control (U) with permanent exclosure cages. The experiment was arranged in four complete randomized blocks (four treatments  $\times$  four replicates = 16 plots) with individual plot sizes of  $1 \text{ m} \times 1 \text{ m}$ . All grazing treatments were grazed by young heifers. The samples  $(0.1 \text{ m} \times 0.1 \text{ m})$  of above-ground biomass were collected by cutting at ground level monthly from May to December (2 samples per plot  $\times$  4 treatments  $\times$  4 replications  $\times$  8 months; in total 256 samples). The biomass samples were sorted into individual vascular plant species and dead material and then dried (at 80°C) to constant dry matter content, and then weighed. Biomass data were recalculated into kg of DM (dry matter) per m<sup>2</sup>. Based on descriptions of vascular plants in the regional flora (Kubát et al., 2002), all plant species within the study area were a priori categorized according to their main traits (derived from previous studies, e.g. Pavlů et al., 2007): tall grasses, short grasses, tall forbs, short forbs and legumes. The proportion of dominant species, dead material and functional groups were analysed by ANOVA. Redundancy analysis (RDA) in CANOCO package, followed by a Monte Carlo permutation test was used to evaluate trends in plant species composition under different treatments.

#### **Results and discussion**

There were significant differences between treatments, as well as successional development, independent of the treatments (see Table 1, analysis A1 and A2 for details). However, the results of RDA analyses showed similarity of treatment 4R to C with highest grazing intensity. When the botanical observations are conducted during the vegetation season, the time is usually revealed as a significant factor affecting sward structure (Kohler *et al.*, 2004). As well as in our study, the time explained two times more plant species variability than applied treatments. It is usually explained by phenological complementarity of the dominant plants species in temperate grasslands (Mládek and Juráková, 2011). However this complementarity was not revealed in our study. The fastest response to different management was with tall and short grasses where the significant differences (P < 0.001) were revealed in June sampling. Not surprisingly, tall forbs (e.g. Gallium album, Hypericum maculatum) had a higher occurrence in U treatment whereas short forbs (e.g. Cerastium holosteoides, Alchemilla sp., Veronica chamaedrys) and mosses occurred mostly in frequently grazed ones (C and 4R). The accumulation of dead material differentiated between treatments since August sampling and reflected the grazing intensity C < 4R < 2R < U (Figure 1a). Contrary to our results from previous studies (e.g. Pavlů et al., 2007) the dominant short grass A. capillaris had higher biomass proportion in U and 2R treatments (Figure 1b). It is because this species, adapted to previous heavy grazing, was allowed to grow with low defoliation frequency (2R) or even without defoliation (U).

Table 1. Results of RDA analyses. Abbreviations: % expl. = explained by axis 1 (all ordination axes) – measure of explanatory power of the explanatory variables; F-ratio = F-statistics for the test of particular analysis; P-value = corresponding probability value obtained by the Monte Carlo permutation test; M = month; C, 4R, 2R, U = treatment abbreviation

Analysis	Explanatory variable	Covar.	% expl.	F-ratio	P-value
A1: Changes in species composition are	M, M $\times$ C, M $\times$ 4R,	DistID	62.2	178.0	0.001
independent on time and on treatments.	$M \times 2R, M \times U$	PIOUD	(62.7)	(45.4)	(0.001)
A2: The short-term changes in species	$M \times C, M \times 4R,$	PlotID,	26.5	39.0	0.001
composition are independent on treatments.	$M \times 2R, M \times U$	Μ	(26.8)	(13.2)	(0.001)



Figure 1. The above-ground biomass of the dominant grass *Agrostis capillaris* (**a**) and of accumulated dead matter (**b**). Significant differences (P < 0.05) with the post-hoc Tukey HSD are indicated with different characters (a-c), n.s. = not significant. C, 4R, 2R, U = treatment abbreviation

## Conclusions

Applied grazing treatments as well as successional development, regardless of treatments, significantly affect the sward structure of upland grassland during the first vegetation season after management introduction. The key factor affecting sward structure during the vegetation season is behaviour of dominant species, plant biomass growth and dead material accumulation.

#### Acknowledgements

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## References

Bullock J.M., Franklin J., Stevenson M.J., Silvertown J., Coulson S.J., Gregory S.J. and Tofts R. (2001) A plant trait analysis of responses to grazing in a long-term experiment. *Journal of Applied Ecology* 38, 253–267. Kohler F., Gillet F., Gobat J.-M. and Buttler A. (2004) Seasonal vegetation changes in mountain pastures due to simulated effects of cattle grazing. *Journal of Vegetation Science* 15, 143–150.

Kubát K., Hrouda L., Chrtek J., Kaplan Z., Kirschner J. and Štěpánek J. (eds) (2002) Klič ke květeně České republiky. [Key to the Flora of the Czech Republic.], Academia, Praha. 928 pp.

Mládek J. and Juráková J. (2011) Using phonological progression and phonological complementarity to reveal potential for late grassland harvest. *Grassland Science in Europe* 16, 139–141.

Pavlů V., Hejcman M., Pavlů L. and Gaisler J. (2007) Restoration of grazing management and its effect on vegetation in an upland grassland. *Applied Vegetation Science* 10, 375–382.

WallisDeVries M.F., Bakker J.P. and Van Wieren S.E. (eds) (1998) Grazing and conservation management. Kluwer Academic Publishes, Great Britain, Dordrecht. 373 pp.

## Measuring grazing intensity in heterogeneous pastures using GPS-tracking

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## Abstract

Grazing intensity is a major determinant of ecosystem services provided by summer pastures. In order to determine local grazing intensity on an alpine farm in Switzerland, we equipped three cows with GPS loggers for the entire 2011 summer season. Position data were recorded every 20 seconds and complemented with information about animal activity (walking, grazing, resting). These were gained through observation of the behaviour of each tagged animal and were then related to the recorded animal movements using classification algorithms. Position data could be classified into the three activity classes of walking, grazing and resting. Grazing intensity estimated with the position data was negatively correlated with slope of terrain. Distant pastures were less visited by the cows than the area around the farm building. Cows frequented the vegetation of *Seslerion* and dwarf shrubs least.

Keywords: animal activity, alpine farm, classification, position data

## Introduction

Grazing influences ecosystem services provided by alpine pastures. In small and topographically uniform paddocks of the lowland, grazing intensity is well approximated by average stocking rate (e.g. Kruess and Tscharntke, 2002). However, this often does not apply to large alpine paddocks, which vary in terms of slope, altitude, stoniness, and vegetation. In this heterogeneous environment, livestock select favoured fodder patches and avoid unsuitable areas (Adler *et al.*, 2001). Jewell *et al.* (2005) found a pronounced small-scale pattern of grazing intensity by recording the position and behavioural activity of cattle on a map several times per day on eight days dispersed throughout the growing season. With the aid of a Global Positioning System (GPS) this study has optimized this method by considerably increasing the recording frequency and period. We investigated the use of GPS loggers as a means to determine local grazing intensity in rough terrain and also investigated the potential use of position data for deriving grazing behaviour. The aim was to predict intensity patterns on extensively managed alpine farms.

## Materials and methods

The summer farm Chlister lies in the Northern foothills of the Swiss Alps at 1700–2100 m a.s.l. From 20 June to 27 August 2011, 35 dairy cows were summered at the farm. The surface area grazed by dairy cows comprised 56 ha, sub-divided into five paddocks of varying sizes (0.7 ha to 27.6 ha). We equipped three cows that were well integrated into the herd with GPS loggers fixed to their collars. The loggers were commercially available low-cost models with improved power supply. Position data were collected every 20 seconds during the whole summer season. For each tracked cow, an observation sequence of several hours was recorded in the field in order to document the activities of grazing, walking, and resting. Behavioural data were aligned to GPS positions (points). We calculated – over one to five positions before and after each point – the accumulated distance along points, the Euclidian distance between points, and the mean values of both measures. A random forest

classifier (Breiman, 2001) was trained first on each observation sequence separately and secondly on all available sequences of the alp Chlister. Based on this classification behavioural activity was predicted for all positions. A kernel-smoothed intensity (Baddeley, 2010) was estimated on the points predicted as grazing. The resulting intensity pattern was correlated to terrain slope data at a 25 m resolution using generalised least squares with a spherical spatial correlation and was compared to the distribution of vegetation mapped at a 50 m resolution.

#### **Results and discussion**

The three GPS loggers at Chlister recorded 250,000 positions over 59 days, corresponding to 84% of the summering time. In an example of an observed track sequence (Figure 1), the oscillation of speed between two subsequent points as measured by the GPS device (top half of Figure 1) show a clear relationship with the observed activities of walking, grazing, and resting (bottom half of Figure 1). Ninety-eight percent of the activity predictions based on this sequence only (bottom half, classified 1), agree with observed activities. If the prediction is deduced from all observed tracks of the alp Chlister (bottom half, classified 2), 96% of points agree, which is a very good basis for a reasonable estimation of grazing intensity. Incorrect classifications occur mostly when activities changed quickly. If so, a recording frequency of 20 seconds is too low, leaving multiple activities occuring between points.



Figure 1. Extract from a track sequence (161 points) with observed and predicted activities. Classified 1: Predictions based on this sequence's data only. Classified 2: Predictions based on all observation sequences from alp Chlister (1847 points)

Figure 2a presents the average stocking rate of the five paddocks at Chlister in contrast to the grazing intensity calculated with the aid of the position data (Figure 2b). Both graphs show a concentration of pasture use around the farm building and less use in the distant areas of the alp. However, local grazing intensity, as measured by the GPS devices, is notably variable within most paddocks. Several exogenous factors influence pasture use by cows, for example pasture management, fodder quality, topography (Adler *et al.*, 2001). Grazing intensity was significantly reduced by slope of the terrain (*Generalized least squares estimate*  $-0.002\pm0.0006^{**}$ ). Pasture use decreased with respect to the vegetation types: *Cynosurion* was the most used, followed by *Nardion* and *Seslerion*, and dwarf shrub was used least.



Figure 2. Area grazed by dairy cows at Chlister. a) average stocking rate of each paddock [LU ha<sup>-1</sup> d], with excluded areas of stable (1), hay meadow (2), and boulder (3), b) grazing intensity derived from GPS positions [LU ha<sup>-1</sup> yr<sup>-1</sup>], c) distribution of slope of terrain at 25 m resolution [%] and d) major vegetation types. A *Cynosurion*, B *Nardion*, C *Seslerion*, D dwarf shrub, E hay meadow (white areas not yet mapped due to time constraints)

#### Conclusions

Position data obtained from GPS tracking can be differentiated into walking, grazing, and resting by using several measures of distance between points for classification. A logging frequency of 20 seconds appears to be a good compromise between data storage constraints and suitable classification of animal activities. The local grazing intensity determined with GPS loggers shows a much more detailed pattern of pasture use by the cows than the average stocking rate of the paddocks. This pattern correlates well with distribution of slope of terrain. The input of additional factors will lead to a better resolution of the intensity pattern and provide information about the effective use of different pasture areas on summer farms.

#### References

Baddeley A. (2010) Analysing spatial point patterns in 'R'. CSIRO online technical publication.

Breiman L. (2001) Random forests. Machine Learning 45, 5-32.

Jewell P.L., Güsewell S., Berry N.R., Käuferle D., Kreuzer M. and Edwards P.J. (2005) Vegetation patterns maintained by cattle grazing on a degraded mountain pasture. *Botanica Helvetica* 115, 109–124.

Kruess A. and Tscharntke T. (2002) Contrasting responses of plant and insect diversity to variation in grazing intensity. *Biological Conservation* 106, 293–302.

Adler P.B., Raff D.A. and Lauenroth W.K. (2001) The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia* 128, 465–479.

## Nitrogen balances for grass-based dairy production systems at different stocking rates

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## Abstract

Nitrogen (N) use efficiency is one of the key drivers of environmentally and economically sustainable agricultural production systems. Loss of N to the environment can result in contamination of waters and can contribute to greenhouse gas emissions. As a result there is a requirement for a balance between N input and N output, while minimising surplus N and N loss. An N-balance model was used to assess the N-use efficiency of grass-based dairy production systems with contrasting stocking rates and N-fertiliser application rates. Data from a three-year grazing study, which had three stocking rates (2.0, 2.47, 2.94 LU ha<sup>-1</sup>) and three N-fertiliser application rates per stocking rate, were used to calculate N-use efficiency and N losses. Nitrogen input was primarily in the form of feed, fertiliser and replacement animals. Milk production was the main source of N output. All stocking rate treatments had similar N-use efficiencies per cow (mean 24.7%). The annual farm gate N surplus per ha ranged from 90 to 171 kg N ha<sup>-1</sup>, 122.8 to 195 kg N ha<sup>-1</sup> and 160 to 233 kg N ha<sup>-1</sup> for the 2.0, 2.47 and 2.94 LU ha<sup>-1</sup> stocking-rate treatments, respectively. As fertiliser N input increased, N surplus per ha increased and N-use efficiency per ha decreased. Increasing stocking rates and maintaining N fertiliser application rate increased N-use efficiency by an average of 22%.

Keywords: N-use efficiency, stocking rate, fertiliser, grazed grass, milk production

## Introduction

Stocking rate (SR) has a profound effect on the productivity of grass-based milk production systems. In a study by Journet and Demarquilly (1979) it was shown that increasing SR by one livestock unit (LU) per hectare (ha) reduced individual animal performance by approximately 10%, while milk production per haincreased by approximately 20%. Similarly, in a meta-analysis of 25 previous studies McCarthy et al. (2010) found that milk yield per cow was reduced by 8%, while milk output per ha was increased by 20% for a one-cow increase in SR. The optimum SR is that which gives the maximum sustainable economic output of product per unit area. Increasing SR increases output per ha, but also requires increased inputs of feed and fertilizer. Intensive grass-based dairy farming relies on fertiliser nitrogen (N) to produce sufficient feed in the form of grazed grass to sustain high milk output per ha at economically attractive levels (Jarvis et al., 1996). Nitrogen-use efficiency is one of the key drivers of the environmental and economic sustainability of dairy production systems. Nitrogen loss to the environment can result in contamination of waters and contribute to greenhouse gas emissions. Achieving the optimum balance between profitable agriculture and environmental sustainability is challenging. When production is maximised and output is near equilibrium, all further N inputs are lost to the environment (Rotz et al., 2005). Nitrogen balances demonstrate the physical difference between the N inputs and N outputs of an agricultural system, with the difference represented by the N surplus (or deficit) (Ledgard et al., 1997). The objective of this study was to assess the N-use efficiency of grass-based dairy production systems with contrasting stocking rates (SRs) and N fertiliser application rates using an N balance model developed by Ryan *et al.* (2011).

## Materials and methods

The physical performance data were obtained from a 3-year study (2007–2009) conducted at the Dairygold Research Farm, Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland (50°07' N, 8°16' W; 46 m above sea level). The soil type is a free-draining brown earth soil of sandy loam to loam texture. The study consisted of three SRs (2.0, 2.47 and 2.94 LU ha<sup>-1</sup>) with three different rates of fertiliser application at each SR. The three annual N fertiliser rates applied to the 2.0 LU ha<sup>-1</sup> treatment were 125, 165 and 205 kg N ha<sup>-1</sup> year<sup>-1</sup>; at the 2.47 LU ha<sup>-1</sup> stocking rate N fertiliser application rates were 165, 205 and 245 kg N ha<sup>-1</sup> year<sup>-1</sup>, and at the 2.94 LU ha<sup>-1</sup> stocking rate the N fertiliser application rates were 205, 245 and 285 kg N ha<sup>-1</sup> year<sup>-1</sup>. Each treatment was managed as an individual farmlet, with its own distinctive area adjusted in size appropriate to the SR, with five cows per treatment. All cows received similar quantities of concentrate (387 kg cow<sup>-1</sup> yr<sup>-1</sup>). Cows were turned out to grass in February post-calving, and they remained at grass until housing in mid-November. The Moorepark Dairy Systems Model (MDSM; Shalloo et al., 2005) used the grazing management data, sources and quantities of N inputs, and types and quantities of N outputs from each treatment to simulate the performance of the dairy production systems. The production data were then used in an N-balance model (Ryan et al., 2011) to calculate N-use efficiency, N surplus and N loss.

## **Results and discussion**

Total N input increased as both SR and N fertiliser application rate increased (Table 1). Milk was the main source of N output, increasing as SR increased (Table 1). All SR treatments had similar N-use efficiencies per cow (24.7%). The annual farm gate N surplus ranged from 90 to 171 kg N ha<sup>-1</sup>, 123 to 195 kg N ha<sup>-1</sup>, and 160 to 233 kg N ha<sup>-1</sup> for the 2.0, 2.47 and 2.94 LU ha<sup>-1</sup> SR treatments, respectively (Table 1). As fertiliser N input increased, the N surplus ha<sup>-1</sup> increased and N-use efficiency ha<sup>-1</sup> decreased. Increasing SR and maintaining N fertiliser application rate increased the N-use efficiency by an average of 22%. At the same SR, the N surplus increased with increasing N input, and N-use efficiency decreased. It has traditionally been suggested that extensive, low-N input, dairy production systems are more environmentally sustainable. However, if the N within the system is not being utilised efficiently losses will continue to occur. This situation was evident when surplus N or N-use efficiency estimates were compared. Nitrogen-use efficiency increased as SR increased, but there was substantial variation between systems stocked at the same SR. The variation in N surplus was greatest between the systems stocked at 2 LU ha<sup>-1</sup>, where production remained similar. Nitrogen-use efficiency was greater when stocking rate was increased while N fertiliser application rate remained the same, for example the treatment stocked at 2.47 LU ha-1 receiving 205 had a greater N-use efficiency than the treatment stocked at 2 LU ha<sup>-1</sup> receiving 205 kg N ha<sup>-1</sup>. Surplus N per kg of milk solids produced was lower at higher stocking rates within the same N fertiliser application rate.

Strategies to improve N-use efficiency can be implemented, depending on the emphasis of the production system (optimising N input, N output or N efficiency). If the emphasis is on output, N efficiency can be improved by higher N output in animal products at the same N input level. Alternatively, N efficiency may be improved through reduced N inputs while maintaining the same animal output. In this study, reducing the N-fertiliser application rate by 40 kg N ha<sup>-1</sup> within the same SR reduced the surplus N per kg milk solids produced by

up to 51%. Alternatively, increasing SR by 0.5 LU ha<sup>-1</sup> and maintaining N-fertiliser application rate reduces surplus N per kg milk solids produced by up to 41%, achieved through increased use of grazed grass, which has been proven to be the most economical method of milk production in Ireland (Shalloo *et al.*, 2004; Kennedy *et al.*, 2006).

Table 1. Milk solids production per hectare and annual farm gate N balance for the nine dairy production systems

Stocking rate	2	LU ha	-1	2.4	7 LU ŀ	$a^{-1}$	2.9	4 LU ł	na <sup>-1</sup>
Fertiliser N application (kg N ha <sup>-1</sup> )	125	165	205	165	205	245	205	245	285
Milk solids yield (kg ha <sup>-1</sup> )	777	752	755	906	975	996	987	1160	1088
Fertiliser application rate (kg N ha <sup>-1</sup> )	124	164	204	164	204	243	204	243	283
Concentrate N consumed (kg N ha-1)	16.8	16.4	17.3	20.7	20.8	21.1	25.2	25.2	25.4
N input in replacements (kg N ha <sup>-1</sup> )	3.4	3.4	3.4	4.3	4.3	4.3	5.0	5.0	5.0
Total kg N input (kg N ha <sup>-1</sup> )	153.1	192.5	233.2	197.6	237.8	277.8	243.1	282.8	322.9
Milk N (kg N ha <sup>-1</sup> )	55.6	53.8	54.3	65.8	71.6	73.5	71.6	83.2	78.5
Calf N (kg N ha <sup>-1</sup> )	2.3	2.3	2.3	2.8	2.8	2.8	3.3	3.3	3.3
Cull cow N (kg N ha <sup>-1</sup> )	5.2	5.2	5.2	6.5	6.5	6.5	7.5	7.5	7.5
Total N output (kg N ha <sup>-1</sup> )	63.1	61.2	61.7	75.1	80.9	82.9	82.4	93.9	89.3
Nitrogen surplus (kg N ha <sup>-1</sup> )	90.0	131.3	171.5	122.8	156.9	195.0	160.8	188.9	233.7
Nitrogen-use efficiency (%)	41.2	31.8	26.5	38.0	34.0	29.8	33.9	33.2	27.6
Surplus N (kg N per kg MS)	0.12	0.17	0.23	0.14	0.16	0.20	0.16	0.16	0.21

#### Conclusions

Increasing SR and N-fertiliser application rate increased the production per ha of milk solids. Nitrogen-use efficiency declined as N-fertiliser application rate increased. Nitrogen surplus per ha increased as SR and N-fertiliser application rate increased. However, increasing SR within a given fertiliser N rate can reduce surplus N. The system stocked at 2.94 LU ha<sup>-1</sup> receiving 245 kg N ha<sup>-1</sup> had a similar N surplus per kg milk solids produced to treatments stocked at 2 and 2.47 LU ha<sup>-1</sup>, while having greater production of milk solids, which contributes to increased economic return per ha. These results suggest that product produced per kg of surplus N can be used as a benchmark for efficient production systems.

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## References

Jarvis S.C., Wilkins R.J. and Pain B.F. (1996) Opportunities for reducing the environmental impact of dairy farming managements: a systems approach. *Grass and Forage Science* 51, 21–31.

Journet M. and Demarquilly C. (1979) Grazing. In: Broster W.H and Swan H. (eds) *Feeding strategy* for the high yielding dairy cow, EAAP publication no. 25, Granada Publishing, London, UK, pp. 295–321. Kennedy E., O'Donovan M., Murphy J.P., O'Mara F.P. and Delaby L. (2006) The effect of initial spring grazing date and subsequent stocking rate on the grazing management, grass dry matter intake and milk production of dairy cows in summer. *Grass and Forage Science* 61, 375–384.

Ledgard S.F., Penno J.W. and Sprosen M.S. (1997) Nitrogen balances and losses on intensive dairy farms. In: *Proceedings of the New Zealand Grassland Association*, No. 59, New Zealand Grassland Association, pp. 49–53. McCarthy B., Delaby L., Pierce K.M., Journot F. and Horan B. (2010) Meta-analysis of the impact of stocking rate on the productivity of pasture-based milk production systems. *Animal* 5, 784–798.

Rotz C.A., Taube F., Russell M.P., Oenema J., Sanderson M.P. and Wachendorf M. (2005) Whole-Farm Perspectives of nutrient flows in agriculture. *Crop Science* 45, 2139–2159.

Ryan W., Hennessy D., Murphy J.J., Boland T.M. and Shalloo L. (2011) A model of nitrogen efficiency in contrasting grass based dairy systems. *Journal of Dairy Science* 94, 1032–1044.

Shalloo L., Dillon P., Rath M. and Wallace M. (2004) Description and validation of the Moorepark Dairy System Model. *Journal of Dairy Science* 87, 1945–1959.

## Grazing preferences of goats in diverse rangeland

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## Abstract

Norwegian goat milk production is based on summer grazing on diverse forest or alpine rangeland, and the quality of these pastures is important for milk quantity and quality. We used *n*-alkanes and long chained alcohols found in plant waxes as markers to estimate diet composition in goats grazing on a heterogeneous rangeland during two periods in summer; early (beginning of July) and late (end of August). Some of the goats were fitted with GPS collars that recorded their position. Preliminary results show a diverse diet, where ferns, sedges, blueberry (*Vaccinium myrtillis*) and birch (*Betula pubescens*) were preferred in early summer. In late summer the diet was particularly diverse, coinciding with a general decline in plant quality.

Keywords: diet preferences, goats, nutrient content, plant wax markers, rangeland

## Introduction

Norwegian dairy goats are fed indoors during winter and released to graze on natural mountain or forest pastures in spring or early summer. They spend on average 125 days and produce about 35–45% of the milk on pastures (TINE, 2006). Farmers are advised to give extra supplement of feed in the form of cultivated pasture, silage or concentrates when the quality of the natural pastures decline, but knowledge is limited on which plant species are grazed, and how the feed quality of grazed herbage, trees and shrubs changes during the season. Generally, the nutritional value of the grazing plants decreases during the grazing period with increased fibre content and decreased content of protein, and the goats try to optimize the nutritional value of their diet by selection of plant species with the highest nutrient concentration (Narjisse, 1991).

## Materials and methods

Goats, about 100 animals, were released on a heterogeneous rangeland pasture (298 ha in total) at Senja, Norway (N 69°21.397', E 17°56.319') in early July, and were allowed to graze day and night. The vegetation of the rangeland was mapped (Bjørklund, 2010). Nine goats were dosed synthetic even-chained alkane C32 twice daily for 12 days. Faeces samples were collected from nine goats during the five last days of the alkane-dosing period and bulked. Grazed plants were sampled simultaneously, and plants and faeces were analysed for alkanes and alcohols. The same procedure was repeated at the end of the grazing season in late August. Plant species were grouped according to alcohol and *n*-alkane profiles using PCA analysis. Their contribution to faecal profiles and diet composition was estimated using least square optimisation. Eight of the goats dosed alkane marker were fitted with GPS collars (Telespor "Radiobjella") in each period, and their positions were recorded every 15 minutes. The data was used to construct a map where we could calculate time spent in different vegetation types and walking distance. Plant nutrient content of crude protein (CP) and neutral detergent fibre (NDF) was analysed with wet chemical methods by Dairy One Coop. Inc. (Ithaca, NY, USA).

### **Results and discussion**

Goats spent most time in the blueberry-birch forest (Table 1), which was the dominant vegetation type in the area. The area was dominated by birch (Betula pubescens), bilberries (Vaccinium myrtillus), Swedish cornel (Cornus suecica), wavy hair-grass (Avenella flexuosa) and sweet vernal grass (Anthoxanthum odoratum). This is also reflected in their diet preferences (Table 2). The vegetation type 'pasture' is dominated by grasses or herbs tolerant to grazing. Although it constituted less than one per cent of the rangeland, the goats spent about one quarter of their time there. However, the GPS data do not indicate whether the goats were grazing or resting. In contrast, the fen constituted almost 18% of the area, but the goats only spent 1-2% of their time there. This vegetation type is dominated by sedges, and the goats grazed especially common cottongrass (Eriophorum angustifolium) and different Carex spp. while passing through the fens on their way to more preferred areas (Helgesen, 2011). Generally, in the first period the goats tended to graze closer to the milk stall. This was reflected in their average walking distance, which was 5.2 km day-1 in the first period and 7.6 km day-1 in the second period (data not shown). They also utilised the meadow-birch forest differently in the two periods. This vegetation is heterogeneous, but characterised by birch forest with an understory of nutrient-demanding herbs, grasses and ferns. The goats spent more time here in the first period and it seemed the areas dominated by ferns were preferred, which is reflected in their diet preferences (Table 2). The 'pasture-land forest' covered a very small area, but was used as resting area in the second period, explaining the high frequency of observations there.

		Average percentage time spent by goats				
Vegetation types	Percentage of total area	in each v	egetation type			
		P1	P2			
Blueberry-birch forest	31.7	37	49			
Pasture, grasses and herbs	0.97	27	23			
Meadow-birch forest	26	19	8			
Pasture-land forest	0.3	2	14			
Lichen/heather-birch forest	6.9	6	3			
Fen	17.6	2	1			
Other	15.7	6	2			
Total area	298 ha					

Table 1. Coverage of vegetation types in per cent of total area in a rangeland grazed by dairy goats in summer 2010. Percentage of time goats spent in different vegetation in early July (P1) and late August (P2)

The diets of the goats were diverse and varied between the individual goats and between the two periods (Table 2). Goats grazed ferns only in early summer, while about one third of the diet was rush and sedges in both periods. In early June goats grazed mainly *Carex* spp. while they consumed more rush and deergrass (*Trichophorum cespitosum*) later in the autumn. They also grazed more grasses, trees and shrubs in late August than earlier in the summer. Especially rowan (*Sorbus aucuparia*) was preferred in the second period. Goats are fond of bark and were observed to consume large amounts despite its low nutritional value.

Blueberry leaves were grazed extensively in early summer, but not later. Generally, the diet was more diverse in the second period, probably reflecting both the availability of preferred plants and a decline in protein content and increase in NDF content. Goats tend to select plants and plant parts with the highest nutrient concentration (Narjisse, 1991).

The *n*-alkane and long-chain-alcohol method to estimate diet composition showed to a certain degree consistency with visual observation of plant preferences. However, in a rangeland as species-diverse as the present, the estimates must be handled with care as the more components included the less reliable results (Dove and Mayes, 2005).

		Diet composition, per cent of					Nutrient content of grazed			
					plants					
Type	Species	D	01	DO		CP, g kg <sup>-1</sup>		NDF	NDF, g kg <sup>-1</sup>	
		г	1	г	2	DM		Ι	DM	
		Mean%	S.E.M.	Mean%	S.E.M.	P1	P2	P1	P2	
Ferns	Athyrium spp., Phegopteris connectilis	11.7	5.31	ND	_	118	106	281	255	
Rush and sedges	Luzula muliflora	ND	_	16.4	2.84	109	71	638	657	
	Carex spp., Eriophorum angustifolium	25.7	0.03	ND	_	144	93	589	628	
	Trichophorum cespitosum	5.19	2.18	18.9	1.84	164	112	583	571	
Grasses	Anthoxanthum odoratum, Avenella flexuosa, Phleum alpinum	6.02	2.30	20.02	2.11	118	79	676	596	
	Trees (Birch bark, Juniper- is communis, Picea abies)	11.3	0.83	1.09	0.80	91	61	441	534	
chrubs	Betula pubescens	13.8	1.30	10.4	0.99	133	105	279	371	
sinuos	Sorbus aucuparia	ND	_	27.9	3.48	99	87	338	447	
	Salix ssp.	2.21	0.95	4.99	1.55	160	135	241	285	
Heath	Vaccinium myrtillus, V. vitis-idea	24.1	1.86	0.29	0.29	88	79	314	343	

Table 2. Estimates of diet composition of dairy goats and nutrient content of grazed species in rangeland in early July (P1) and late August (P2)

<sup>1</sup>Proportion of total diet – each value is the mean of nine goats, S.E.M. standard error of the mean, DM: dry matter, CP: crude protein, NDF: neutral detergent fibre, ND: not detected.

#### Conclusions

Goats grazed a very diverse diet, especially late in autumn, and the species preferences coincided to some degree with nutrient content of the plants. They utilised different areas of the rangeland in the two periods, and had longer walking distance in late autumn compared with early summer.

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#### References

Dove H. and Mayes R.W. (2005) Using n-alkanes and other plant wax components to estimate intake, digestibility and diet composition of grazing/browsing sheep and goats. *Small Ruminant Research* 59, 123–139. Helgesen R.M. (2011) *Changes in the grazing preferences and feed intake of goats during the grazing season.* Master Thesis, Norwegian University of Life Sciences, Department of Animal and Aquacultural Sciences, 64 pp.

Narjisse H. (1991) Feeding behaviour of goats on rangelands. In: Morand-Fehr P. (ed.), *Goat Nutrition*. EAAP Publication No 46, 1991, Pudoc Wageningen, pp. 13–24.

Bjørklund P. (2010) Vegetasjon og beite i Svartfjell geitbeite. Rapport frå Skog og landskap no 5/05. The Norwegian Forest and Landscape Institute, Ås 35 pp.

TELESPOR. *Info* [Online]. Available: http://telespor.no/?page\_id=368 [Accessed 21.02.112011]. TINE, 2006, Strategiplan for geit i TINE 2006–2010.

# Tree and meadow production under birch silvopastoral system: fertilization, seed mixture and tree density effect

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## Abstract

The combination of tree and pasture production has been recently promoted by the European Union as an agroforestry system type. Pasture production under trees produces annual farm outputs which promote long term rural population stabilisation compared with exclusively forest systems. At the same time, the presence of a tree increases the long term value of the land. The objective of this study was to evaluate the effect of two different tree densities, seed mixtures and fertilization on annual meadow production after 9 years of a silvopastoral system. The tree species was birch (Betula alba L.) established at two densities (2500 and 833 tree ha<sup>-1</sup>). Fertilization regimes included no fertilization and mineral inputs. Initial seed mixtures included cocksfoot (Dactylis glomerata) and perennial ryegrass (Lolium perenne) together with white and red clover (Trifolium repens and T. pratense). The results showed a significant effect of fertilization, seed mixture and tree density on meadow production. Meadow production was not reduced over time in spite of tree canopy development, which makes birch more suitable for meadow production when compared with radiate pine (Pinus radiata). Fertilization had a major effect at the start of the experiment in terms of modifying annual meadow production. However, by the end of the experiment its effect was modified by tree density and previous seed mixture.

Keywords: agroforestry, nitrogen, understory, light, botanical composition

## Introduction

Agroforestry systems are promoted by European Union as this land management option is suitable for receiving direct payments at establishment according to the last Rural Development Regulation (EAFRD, 2005). Silvopastoral practices are the most extensive form of agroforestry system in Europe (Mosquera-Losada *et al.*, 2011) and pasture production depends on key tree species and their density. *Betula alba* L. (birch) is quite suitable for supporting high pasture production compared with other widely used tree species like *Pinus radiata* D. Don, due to the low leaf density of their tree canopy, but also because they are deciduous, which allows light to reach the understory and therefore permits photosynthesis. Light is the main factor limiting understory production and its fertilization response in silvopastoral practices, and the shade-tolerant species could appear, increasing pasture production. This study aims at evaluating the effect of birch density, fertilization and sown pasture mixture on meadow production over a nine-year period.

## Materials and methods

The experiment was established on agriculturally abandoned land in Castro de Riberas de Lea (Lugo, Galicia, NW Spain) in 1995, at an altitude of 439 m above sea level. The experimental design was a randomized complete block with three replicates and eight

treatments. Treatments consisted of two birch densities (833 and 2500 trees  $ha^{-1}$ ), two fertilization types (No fertilization and Mineral fertilization) and two seed mixtures (cocksfoot cv Artabro (25 kg ha<sup>-1</sup>) + white clover cv Ladino (4 kg ha<sup>-1</sup>) + red clover cv. Marino  $(1 \text{ kg ha}^{-1})$  and perennial ryegrass cy Brigantia  $(25 \text{ kg ha}^{-1})$  + white clover cy. Ladino  $(4 \text{ kg ha}^{-1}) + \text{red clover cv Marino} (1 \text{ kg ha}^{-1}) \text{ established in spring 1995. Each experimen$ tal unit had 25 trees planted with an arrangement of  $5 \times 5$  stems, forming squares of area  $192 \text{ m}^2$  and  $64 \text{ m}^2$  for the low and high tree density, respectively. Pasture was sown at the start of the experiment and the Mineral treatment consisted of annual inputs of 40 kg N ha<sup>-1</sup>, 52.4 kg P ha<sup>-1</sup> and 66 kg K ha<sup>-1</sup>). Meadow production was harvested four times every vear (three in spring and one in autumn), unless meadow production was too low to perform the harvest. Meadow production was measured by harvesting an area between the 6 central trees of each experimental unit. A sample was transported to the laboratory to estimate dry matter (48 hours at 60°C). The meadow production of each harvest was summed to obtain annual production. In this study only 6 years are shown. Meadow production was analysed every year with an ANOVA (proc glm procedure) and, if significant, means were separated by using Duncan's test.

#### **Results and discussion**

Annual meadow production is shown in Figure 1. Meadow production was usually below 7 t  $ha^{-1}$ , which is within the normal range of values usually found in the area (Mosquera-Losada et al., 1999). With the exception of the first year, the treatments affected annual meadow production. In the first year the meadow was sown in spring, and annual species colonized the sward, which limited the response to treatments. During the first years, meadow production clearly responded to fertilization. In 1996, annual meadow production was higher if mineral fertilizer was added to the soil compared with the lack of fertilization, as has been noted in many studies (Whitehead, 1995). A similar response was found in 1998, but it was only significant at high densities. However, the fertilization effect was only found at low densities in 2000 and 2002, when the response to this fertilizer was better if a cocksfoot had been previously sown, as the establishment of ryegrass was poorer (Mosquera-Losada et al., 2006). However, in the last year of the experiment fertilization increased annual meadow production only in the ryegrass mixture, compared with the treatments without fertilization and sown with either ryegrass or cocksfoot. Cocksfoot proportion in the experiment was very high in DG sown treatments, which could have limited the establishment of other species and therefore to leave gaps not easy to colonize.

Meadow production was higher under birch than that found under *Pinus radiata* D. Don in a similar experiment (Fernández-Núñez *et al.*, 2010), which is explained by the high amount of light reaching understory if birch is used when afforestation is carried out. Moreover, this also explains why mineral fertilizer produces a meadow response even after nine years of the experiment. However, the size of the response depended on the seed mixture. Cocksfoot persistence after 6 years was better than ryegrass, which increased its response to fertilization. However, after 9 years of the experiment the cocksfoot proportion was equally as low as ryegrass, which permitted weeds to develop and be able to grow up better under fertilization conditions, thereby increasing meadow production.



Figure 1. Annual meadow production at two densities (833 and 2500 trees ha<sup>-1</sup>), sown with cooksfoot (DG) or ryegrass (LP) meadow mixtures with mineral fertilizer or without fertilization (NF). Different letters indicate significant differences between treatments within each year. \*\*\* P < 0.001; \* P < 0.05; ns: not significant

#### Conclusions

Birch is an adequate species to establish in silvopastoral practices, as it allows meadow growth and response to fertilization as the light reaching the understory is enough to allow this. However, the species sown should be adequate to develope under shaded conditions.

## References

EAFRD (2005) Council reculation N° 1698/2005 of 20 September 2005 on support for rural development by the European Agricultural fund for rural development. Official Journal of the European Union L277/1. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:277:0001:0040:EN:PDF Accessed 17 May 2011.

Fernández-Núñez E., Mosquera-Losada M.R. and Rigueiro-Rodríguez A. (2010) Carbon allocation dynamics one decade after afforestation with *Pinus radiata* D. Don and *Betula alba* under two stand densities in NW Spain. *Ecological engineering* 36, 876–890.

Mosquera-Losada M.R., Moreno G., Pardini A., McAdam J.H., Papanastasis V., Burgess P.J., Lamersdorf N., Castro M., Liagre F. and Rigueiro-Rodríguez A. (2011) Past, present and future of agroforestry systems in Europe. *Advances in Agroforestry* (in press).

Mosquera-Losada M.R., Rigueiro-Rodríguez A. and González-Rodríguez A. (1999) Ecología y manejo de praderas. Ministerio de agricultura, pesca y alimentación. Madrid.

Whitehead D.C. (1995) Grassland nitrogen. CAB international, Wallingford.

# Habitat selection in old and modern dairy cattle breeds on Norwegian mountain pastures

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## Abstract

The aim of this study was to investigate the habitat selection in old and modern dairy cattle breeds on mountain pastures in Central Norway. Two neighbouring dairy cattle herds in a summer farming landscape were studied by GPS-tracking. The GPS-tracking data and vegetation maps were used to calculate home range size and habitat selection ratio. The modern cattle breeds had a larger home range than the old breeds, and there was an overall habitat selection within both groups.

Keywords: dairy cattle, habitat selection, grazing, mountain pastures, breeds, home range

## Introduction

Many farmers keeping the old breed Blacksided Trønder and Nordland Cattle (STN) are of the opinion that this breed has traits of importance for extensive production systems (Sæther and Vangen, 2001). Results from a study of two dairy breeds (STN and the modern Norwegian Red, NRF) grazing semi-natural grasslands in the Norwegian mountains also indicate that there may be differences in plant and vegetation preferences between moderate and lower yielding breeds (Sæther *et al.*, 2006; cf. also Rook *et al.*, 2004). However, so far, studies of differences between old and modern cattle breeds with regard to grazing preferences on semi-natural grasslands are few. On this background, a study of habitat selection of old and modern breeds grazing on semi-natural grasslands was carried out.

## Materials and methods

The grazing behaviour of two neighbouring dairy cattle herds in the summer farming landscape in Budalen, Central Norway, were studied by GPS-tracking for every minute (8–10 hours per day) and field studies by focal sampling for 30 minutes per individual animal, during five days in 2009. Five cows of each herd wore GPS-receivers during the day. One of the herds consisted of NRF cattle (modern breed) whilst the other was a mixed herd with old cattle breeds (local names: Vestlandsk Fjordfe and Sidet Trønder- og Nordlandsfe, STN). Grazed areas were situated 650–900 m above sea level in the sub-alpine birch woodland. Detailed vegetation maps for both study areas were prepared by interpretation of aerial photos and field work. An intersect analysis in ArcMap 9.3 (ESRI) was used for computing a geometric intersection of GPS-positions (of individual cattle) and vegetation types.

Home range was calculated for each individual (n = 10) by the use of 95% minimum convex polygon (MCP). The difference between home range sizes between breed groups were tested by ANOVA. Habitat selection was analyzed by calculating habitat selection ratio (SR) (Manly *et al.*, 2002). Habitat types with SR > 1 and confidence intervals (CI) values not including the value one is selected for, whereas SR < 1 where CI does not include the value one is selected against (Manly *et al.*, 2002). The available area of habitat types was different between the breed groups. Hence, SR was calculated for each breed group separately. The package 'adehabitat' (Calenge, 2006) in R for windows version 2.12.2 was

used for calculating home range size and selection ratio with related chi-square tests (R Development Core Team, 2008).

### Results

The modern breed had a larger home range (1.34 km<sup>2</sup> ± 0.23 SD) than the old breed group (0.32 km<sup>2</sup> ± 0.06 SD; F = 80.14, *P* < 0.001. It was individual differences in habitat selection within both the old ( $\chi^2$  = 299.335, *P* < 0.001) and the modern herd ( $\chi^2$  = 408.52, *P* < 0.000). In both breed groups there was a strong overall habitat selection (traditional breed;  $\chi^2$  = 20510.09, *P* < 0.001, modern breed;  $\chi^2$  = 6420.98, *P* < 0.001).

SR varied between breed groups. Semi-natural pasture and road were selected by both breed groups (Figure 1). In addition, the modern breed group selected overgrown summer farm hay meadow, but this habitat type was not available for the old breed herd. Waiting place/ semi-natural pasture had high SR values for both breed groups but within the modern breed group the CI interval was wide due to individual variation in SR values. The old breeds did not select river/riverbank, grass-rich sub-alpine birch woodland, intermediate fen, or intermediate wooded and scrub-covered fen. The modern breed also had low SR values for intermediate wooded and scrub-covered fen and river/riverbank and in addition dwarf birch/willow heath and low alpine vegetation zone.



Figure 1. Global Manly habitat selection ratio (SR) for a modern dairy cow breed (a) and an old dairy cow breed (b) group grazing in a summer farming landscape with the vegetation types dwarf birch/willow heath, grass-rich sub-alpine birch woodland, intermediate fen, intermediate wooded and scrub-covered fen, low alpine vegetation zone, overgrown semi natural meadow, river/riverbank, road, semi-natural pasture, waiting place/semi-natural pasture. SR and CI intervals values above one (the dotted line) indicate preference, whereas values below one indicate avoidance

#### Discussion

The results from this small study show that the cows prefer to use roads when they move around, that they use much time at the pasture/waiting area outside the summer farm and that both studied herds prefer grass- and herb-rich semi-natural pastures as grazing areas. In addition, the modern breed group selected overgrown but still grass-rich summer farm hay meadows, while this habitat type not was available for the old breed herd. Furthermore, the modern breed had a larger home range and a more varied use of habitat types than the old, native breeds. This is not in accordance with results from other similar studies. However, this may be explained by the fact that the mixed herd (with old breeds) has a large seminatural pasture easily available and therefore had less need for other grazing areas. The area around this semi-natural pasture is perhaps also less attractive due to encroachment by trees and bushes. The modern-breed herd has to move around more to find good grazing areas.

#### Conclusions

This study does not support the opinion that old breeds are more adapted to extensive production systems than the modern NRF breed. However, this may be a result depending on the landscape situation more than on differences between breeds.

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## References

Calenge C. (2006) The package 'adehabitat' for the R software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling* 197, 516–519.

Manly B.F.J., McDonald L., Thomas D.L., McDonald T.L. and Erickson W.P. (2002) *Resource selection by animals: statistical design and analysis for field studies.* Kluwer Academic Publishers, Boston.

R Development Core Team (2008) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rook A.J., Dumont B., Isselstein J., Osoro K., Wallis de Vries M.F., Parente G. and Mills J. (2004) Matching type of livestock to desired biodiversity outcomes in pastures – a review. *Biological Conservation* 119, 137–150.

Sæther N.H. and Vangen O. (2001) Motives for utilizing the Blacksided Trønder and Nordland: A native cattle breed in Norway. *Animal Genetic Resources Information* 31, 15–26.

Sæther N.H., Sickel H., Norderhaug A., Sickel M. and Vangen O. (2006) Plant and vegetation preferences for a high and a moderate yielding Norwegian dairy cattle breed grazing semi-natural grasslands. *Animal Research* 55, 367–387.

# Grazing system and botanical diversity effects on the quality of herbage consumed by dairy cows

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## Abstract

Selection and foraging behaviour of 9 Holstein and 9 Monbéliarde dairy cows were compared between continuous (CG) or rotational (RG) grazing management on mountain pastures in the centre of France over a two-week period. The CG plot was a species-rich heterogeneous pasture whereas the RG plot (divided in 2 paddocks) was a old temporary grassland. Botanical composition, pasture structure, stem and leaf proportions, and nutritive value of grazed patches were measured. On the RG system an increase in grazing frequency of shorter patches to the detriment of higher ones was observed, along with a decrease in leaf proportion and in forage quality. In spite of the great botanical diversity, on the CG system, the proportion of forbs and legumes in the cow diet was very small. At the end of the exploitation of CG plot an increase in selection of short selected patches and in their leaf proportion, and consequently in nutritive value, was shown, suggesting that cows select regrowth vegetative patches. The CG system allowed cows to graze herbage with a quite constant nutritive value during the short period, when compared to RG.

Keywords: grazing management, mountain pasture, selection, grazing behaviour, dairy cows

## Introduction

Grazing is a major tool for conservation of extensive pastures in mountain areas and maintenance of their biodiversity. Herbivore grazing selection strongly affects sward dynamics and structure. Cows can consume vegetation homogeneously when their grazing selection is limited (Morris, 2002; Teague and Dowhower, 2003), or re-graze preferred areas continuously on extensive grazing systems, showing 'patch grazing' (Alder *et al.*, 2001; Dumont *et al.*, 2007). Changes in grazing selection along the grazing season have been studied by several authors (Ksiksi *et al.*, 2006; Coppa *et al.*, 2011). However, little is known about the daily variations in dairy cow grazing selection according to the grazing system. The aim of this work was to investigate daily variations in grazing selection by dairy cows under two different grazing systems on upland pastures selected to match typical grazing management found in the region: continuous grazing on high biodiverse pasture (CG) or rotational grazing on less biodiverse pasture (RG).

## Materials and methods

The experiment was carried out at the INRA experimental farm of Marcenat (mountain area of central France) where 9 Holstein and 9 Montbéliarde dairy cows, divided in two equivalent groups, were used. The first group (CG) grazed continuously at medium stocking density (2.5 LU ha<sup>-1</sup>) a species-rich heterogeneous pasture (9.6 ha; 139 species); the second one (RG) grazed by rotational grazing at higher stocking density (3.75 LU ha<sup>-1</sup>) on two paddocks constituted by an old temporary, less-diversified grassland (a total of 6.4 ha, 65 species). Their botanical composition was determined using 40 and 33 surveys (Braun-Blanquet, 1932), on CG and RG, respectively. The main species on CG plot were *Agrostis capillaris* (17.2%), *Trifolium repens* 

(12.5%), *Festuca* gr. *rubra* (11.3%), *Achillea* gr. *millefolium* (5.2%), and *Plantago lanceolata* (4.6%), whereas on the RG paddock 1 the main species were *Trifolium repens* (17.5%), *Lolium perenne* (14.2%), and *Festuca* gr. *rubra* (10.4%), and on paddock 2 *Dactylis glomerata* (15.0%), *Agrostis capillaris* (15.0%), *Festuca* gr. *rubra* (14.2%) and *Trifolium repens* (10.8%). Grazing selection was characterized by direct observations and simulated bites, sampling at the beginning and at the end of each RG paddock exploitation on both grazing systems. The grazing cows were observed continuously during the whole day by an observer standing within 5 m of the side of target animal (Farruggia *et al.*, 2008). The observer recorded the composition and structure of one-on-five patches grazed by the target cow, noting the patch height (short/high), phenological stage (vegetative/mature) and the dominant botanical group (grasses/legumes/forbs). Observing the composition and structure of animal bites, the operator sampled a sward patch the more similar as possible to those grazed by the observed cow. Simulated bites were analysed for nutritive value. The dry matter (DM) proportion of the main botanical groups (grasses/legumes/forbs), and DM proportions of leaves/stems of grasses were determined by separating by hand and oven-drying of samples.

#### **Results and discussion**

The high stocking density applied in RG system results in a low species grazing selection. Vegetation was just grazed by layers, starting from the upper one, as already reported by Teague and Dowhower (2003), and by Coppa et al. (2011). This pattern results in a higher selection of high vegetative patches at the beginning of the paddock exploitation, decreasing afterwards (-53.9% on paddock 2; on paddock 1 differences were not significant). In parallel the frequency of selected short vegetative patches dominated by grasses (SGV) increased throughout the paddock exploitation (+11.5% and +48% for the paddocks 1 and 2, respectively; Table 1). The higher proportion of leaves in the upper layer at the beginning of the paddock exploitation explained the higher OMD, and a lower NDF content in the simulated bites  $(-6.3\% \text{ and } -14.2\% \text{ for OMD}, \text{ and } +3.1\% \text{ and } +7\% \text{ for NDF on pad$ dock 1 and 2, respectively; Table 1). These results are in agreement with Abrahamse et al. (2008) who measured the leaves/stems ratio on rotational grazing systems. In our trial, the quality of consumed patches rapidly decreased along the exploitation in parallel with the decrease in proportion of leaves  $(-5.9\%, \text{ and } -10.6\%, \text{ for paddocks } 1 \text{ and } 2, \text{ respec$ tively; Table 1), that were mainly located in the upper sward layers. The described grazing patterns were less evident during the exploitation of paddock 1 when compared to paddock 2, because of its more advanced phenological stage of the sward. At the beginning of exploitation of paddock 1, forage showed a higher NDF content (+3%), and lower ODM (-5%) and leaf proportion (-9.7%), as compared to the beginning of the exploitation of paddock 2 (Table 1). The CG plot vegetation was characterized by high biodiversity and heterogeneity in species distribution, with a low proportion of grasses (on average 51.5% of specific contribution). However, their proportion in the cow diet was very high (on average 91.5% of simulated bites DM). These results are in agreement with Coppa et al. (2011), who showed an exponential relation between the specific contribution and the species DM consumption in a similar grazing system. At the beginning of the CG plot exploitation cows frequented mainly the areas dominated by species with higher nutritive value. The simulated bites had the highest OMD and the lowest NDF content (+8.1% and -4.2% between the beginning and the end of paddock exploitation, respectively; Table 1). Moreover, at the beginning of the plot exploitation, cows selected vegetative patches with higher proportion of leaves than during the middle of exploitation (+16.4%). Our data are in agreement with Coppa et al. (2011), who observed the same selection patterns at the beginning of
the grazing season when dairy cows grazed continuously an extensive pasture. Dumont *et al.* (2007) obtained similar results with beef cattle that grazed continuously on vegetation characterized by a high heterogeneity in species distribution and in nutritive value. Since the plot exploitation progressed, cows overgrazed the preferred and already partially consumed patches and the regrowth present on it, showing the so called 'patch grazing' as described by Adler *et al.* (2001). The proportions of SVG and leaves, related to the regrowth on previously consumed areas, increased at the end of the paddock exploitation (+34.6% and +9.0%; Table 1). On the other hand, the stem proportion of simulated bites was still dominant, and in parallel the average phenological stage of the sward developed, resulting in a slight increase in NDF content and decrease in OMD (+1.3% and -3.8%, respectively; Table 1).

		0	<b>U</b> .									
	RG pac	ddock 1	RG pa	ddock 2		C	G		SEM	Si	gnifican	ice
	D1	D7	D10	D15	D1	D7	D10	D15	SEM	Т	D	D×T
OMD (%)	71.2 <sup>b</sup>	64.9 <sup>cd</sup>	76.2ª	62.1 <sup>de</sup>	67.6 <sup>bc</sup>	63.5 <sup>de</sup>	63.3 <sup>de</sup>	59.5°	0.71	***	***	***
NDF (% of DM)	53.0 <sup>d</sup>	56.1 <sup>bc</sup>	50.6 <sup>d</sup>	57.6 <sup>abc</sup>	55.9°	57.3 <sup>bc</sup>	58.8 <sup>ab</sup>	60.1ª	0.43	***	***	***
Grasses (% of DM)	92.3 <sup>ab</sup>	94.0 <sup>ab</sup>	88.8 <sup>b</sup>	96.9ª	90.6 <sup>ab</sup>	86.8 <sup>b</sup>	94.7 <sup>ab</sup>	94.0 <sup>ab</sup>	0.68	ns	Ť	*
Leaves (% of DM)	31.0	25.1	40.7	30.1	50.1	32.5	34.9	43.9	1.81	**	Ť	Ť
SVG (%)	11.2	22.7	14.0	62.2	43.7	58.3	40.9	75.5	1.91	***	***	ns
HVG (%)	47.3 <sup>b</sup>	46.8 <sup>b</sup>	68.1ª	14.2°	43.1 <sup>b</sup>	16.9°	33.1 <sup>bc</sup>	2.1 <sup>d</sup>	1.70	**	***	***

Table 1. Characteristics of patches selected by dairy cows during the experiment on continuous grazing (CG) and rotational grazing (RG) systems

SEM: standard error of the mean; T: treatment; D: day; OMD: organic matter digestibility; NDF: neutral detergent fibre; SVG: patches dominated by short vegetative grasses; HVG: patches dominated by high vegetative grasses; ns: not significant;  $\dagger: P < 0.1$ ; \*: P < 0.05; \*\*: P < 0.01; \*\*\*: P < 0.001. Means with different superscripts resulted significantly different at P < 0.05.

#### Conclusions

The CG system allowed cows to graze herbage with a quite constant nutritive value during the short period, when compared to RG system, for which wide daily variations in grazed patches and in their feeding quality were observed.

#### References

Abrahamse P.A., Dijkstra J., Vlaeminck B. and Tamminga S. (2008) Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. *Journal of Dairy Science* 91, 2033–2045. Adler P.B., Raff D.A. and Lauenroth W.K. (2001) The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia* 128, 465–479.

Brawn-Blanquet J. (1932) *Plant Sociology*. Fuller G.D., Conard H.S. (eds) McGraw-Hill book company, inc. New York, US, 439 pp.

Coppa M., Farruggia A., Pradel P., Lombardi G. and Martin B. (2011) An improved grazed class method to estimate species selection and dry matter intake by cows at pasture. *Italian Journal of Animal Science* 10, 58–65. Dumont B., Rook A.J., Coran C. and Röver K.U. (2007) Effects of livestock breed and grazing intensity on biodiversity and production in grazing systems. 2. Diet selection. *Grass and Forage Science* 62, 159–171. Farruggia A., Dumont B., D'hour P. and Egal D. (2008) How does protein supplementation affect the selectivity and performance of Charolais cows on extensively grazed pastures in late autumn? *Grass and Forage Science* 63, 314–323.

Ksiksi T., Hirata M., Ogura S. and Fukuyama K. (2006) Effect of patch characteristics on progressive pasture utilization by cattle. *World Journal of Agricultural Science* 2, 162–168.

Morris C.D. (2002) Patch grazing of Tall Grassveld by cattle after a spring burn. African Journal of Range and Forage Science 19, 107–116.

Teague W.R. and Dowhower S.L. (2003) Patch dynamics under rotational and continuous grazing management in large, heterogeneous paddocks. *Journal of Arid Environment* 53, 211–229.

# Effects of environmental factors on yield and milking number in dairy cows milked by an automatic system located in pasture

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## Abstract

A herd of 45 Holstein dairy cows was milked with an automatic milking system (AMS) located on a permanent pasture. The cows grazed from 22.06.2010 until 20.10.2010 on a rotational system. They were fetched twice a day to the AMS but they could also reach it freely. The sward height was measured at the entry and exit of each paddock. Data about daily milk yield and milking number were analysed according to a GLM including the effect of animal, days in paddock, distance between AMS and paddock, rotation cycle number and complementation. The cows produced daily 19.6 kg in 2.1 milkings and 95% of the cows passed at least more than twice a day in the AMS. The milking number decreased when grass height decreased (P < 0.001;  $r^2 = 0.53$ ). The models explained 76 and 28% of the milk yield and milking number variations respectively. Amongst the parameters studied, the animal effect explained 77% of the milk yield and 53% of the milking number variations, respectively (P < 0.001). The distance explained a weak but significant part of the variation in milk yield and milking number (2.3% and 3.8% respectively; P < 0.001). There were no clear relationships between milk yield or milking number and distance.

Keywords: automatic milking system (AMS), dairy cows, grazing, milking number, milk yield

## Introduction

The number of farms equipped with an automatic milking system (AMS) is rising. The use of this system will probably increase in the next years owing to reduced labour costs. In most farms equipped with an AMS, the cows remain in the barn without any grazing opportunity. The benefits from grazing practice, such as reduced feed costs, improved animal welfare and health, are thus lost. Furthermore, grazing is perceived positively by consumers. However, some experiences has proven that grazing is possible with cows milked by an AMS (Spörndly and Wredle, 2005; Davis *et al.*, 2005). Recently, AMS were placed in mobile structures which can be used both indoor and on pasture (Oudshoorn, 2007; Haan *et al.*, 2010). The present trial was carried in a similar manner with a mobile AMS as described by Dufrasne *et al.* (2010). The present study assessed the effects of grass height, animal, days in milk, rotation cycle number and distance from grazed paddocks on daily milk yield and milking number of grazed cows.

## Materials and methods

A herd of 45 Holstein dairy cows of the University of Liège was milked with an AMS the first time on 20.04.2010. The cows grazed from 22.06.2010 until 20.10.2010 on a permanent pasture in a rotational system with 11 paddocks. The flora was composed mainly of grasses (70%) and white clover (15%). The paddocks were cut for grass silage on 15.05.2010. The areas were comprised between 1.33 and 4 ha. The distance between paddocks and the AMS was comprised between 100 m and 425 m. The AMS was located in a paddock of 1.33 ha. In this first year, it was decided to fetch the cows twice a day at 06.00 h. and 16.00 h. to a waiting place with an area of 400 m<sup>2</sup>, in order to pass the cows in the AMS twice per day. Furthermore, the cows had free access to the AMS during the whole day. The cows could see the AMS from 6

paddocks only. The AMS was lit during the night. A drinking bowl was present in each grazed paddock and a water trough was available in the waiting room and at the exit of AMS. At the beginning of grazing period, days in milk were  $172\pm61$  days, animals weighed  $645\pm63.1$  kg, produced  $29.9\pm5.42$  l milk per day and their number of calvings was  $2.3\pm1.15$ . In the AMS, they received on average 2.15 kg concentrate per day with a crude protein (CP) content of 170 g kg<sup>-1</sup> DM. The sward height was measured with a sward stick at entry and exit of each paddock. A grass sample was taken at entry into the paddock in order to determine CP, neutral detergent fibre (NDF) and water soluble carbohydrate (WSC) contents. The cows also received 6 kg DM per cow of maize silage until 08.08.2010, because the grass availability was low due to hot and dry weather. The cows also received a similar amount of maize silage from 11.10.2010 in order to prepare them for the winter diet.

The effect of sward height on mean daily milking numbers was analysed by GLM (31 data). The data about daily milk yield and milking number (4500 data) were analysed with a GLM (SAS, 1999) including the effect of animal, days in paddock, distance, rotation cycle number and complementation. Moreover, the effect of milk yield and milking number were reciprocally inserted in the respective models.

#### **Results and discussion**

From 22.06.2010 until 20.10.2010, the cows produced daily on average 19.6 kg in 2.1 milkings. 95% of the cows passed more than twice a day on at least one occasion during the grazing season. Figure 1 shows the evolution of milk yield during the grazing period. Milk yield decreased as the days in milk increased (289±86 on 20 October) and the diet mainly consisted of grass except the concentrate given in the AMS (Figure 1). Grass CP, NDF and WSC contents were  $232\pm50$ ,  $447\pm63$  and  $73\pm32$  g kg DM<sup>-1</sup>. From these results, it appeared that grass quality was high. The entry and exit grass heights in paddocks were at  $10.2\pm1.9$ and  $3.2\pm1.0$  cm respectively. The mean daily milking number of the herd at entry and exit of each paddock grazed without any maize silage supplementation is shown in Figure 2. The milking number increased when grass heights decreased:

milking number = 2.38 - 0.035 sward heights (cm); P < 0.001,  $r^2 = 0.53$ .

Ketelaar-de Lauwere *et al.* (2000) reported also an increase in milking number when sward heights decreased. Milking numbers of less than twice daily were due to difficulties in bringing some cows to the waiting place when grass availability was high, such as at entries in the new paddock, or to milking failures.

The variation expressed as percentage of the total variation in the statistical models was 76% for milk yield and 28% for milking number. The animal effect explained 77% of the model





Figure 1. Evolution of milk yield during the grazing period

Figure 2. Effects of grass heights measured at entry and exit in the paddock on mean daily milking number



Figure 3. Relationships between the distance from the AMS to paddock and daily milk yield or milking number

variance in milk yield, vs. 53% of the variation in milking number, the effects being significant (P < 0.001). Corresponding values for days in milk were 12% (P < 0.001) and 31% (NS). The lack of significance was probably due to the fact that, at the beginning of the grazing period, most of the cows had calved almost 100 days previously; thus they were past the lactation peak. The days in paddock and distance explained only 2.3 to 3.8% of the variation of both parameters (P < 0.001). There were no clear effects of the distance to the AMS on milk yield and milking number as shown in Figure 3. Ketelaar-de Lauwere *et al.* (2000) observed that a distance up to 360 m between pasture and barn did not affect the visits of cows. By contrast, some authors obtained negative effects of the distance on milking number and, conversely, the milking number was explained at 7.3% by the milk yield (P < 0.001). The rotation cycle number explained only 2.5% of the milk yield model variation (P < 0.001) and 0.5% in the milking number (P < 0.05). The complementation explained 0.5% of the milking number variations (P < 0.05) and a very marginal part of the milk yield (NS).

#### Conclusions

From these results, it appeared that, in a context of milking with an AMS on pasture with cows that were past their lactation peak, the differences in milk yield may be ascribed mainly to an animal effect. The determination of milking number remains largely undefined, although the animal effect appeared also to be important. At up to 450 m, the distance did not seem to influence notably either milking number or milk yield. More studies are needed to analyse the behaviour of the cows milked by AMS on pasture and to find out the means to attract the cows to the AMS.

#### References

Davis K.L., Jago J.G., Wieliczko R., Copeman P.J.A., Bright K. and Woolford M.W. (2005) Factors influencing milk harvesting efficiency in an automatic milking system. *Proceedings of the New Zealand Society of Animal Production* 65, 271–274.

Dufrasne I., Robaye V., Istasse L., and Hornick J.-L. (2010) Grazing with a mobile milking robot. *Grassland Science in Europe* 15, 217–219.

de Haan M.H.A., Philipsen A.P., Van den Pol-van Dasselaar A., Holsof G., Lenssinck F.A.J. and de Koning C.J.A.M. (2010) Better grazing opportunities with a mobile milking robot. *Grassland Science in Europe* 15, 178–180.

Ketelaar-de Lauwere C.C., Ipema A.H., Lokhorst C., Metz J.H.M., Nordhuizen J.P.T.M., Schouten W.P.G. and Smits A.C. (2000) Effect of sward height and distance between pasture and barn on cows visit to an automatic milking system and other behaviour. *Livestock Production Science* 65, 131–142.

Oudshoorn R.W. (2007) Mobile milking robot offers new grazing concept. *Grassland Science in Europe* 13, 721–723.

Spörndly E. and Wredle E. (2005) Automatic milking and grazing – Effects of location of drinking water on water intake, milk yield and cow behaviour. *Journal of Dairy Science* 88, 1171–1722.

Wredle E. (2005) Automatic milking and grazing: factors and stimuli affecting cow motivation to visit the milking unit. Thesis, Swedish University of Agricultural Science, Uppsala, 46 pp.

# Palatability of herbage, and woody plant encroachment in relation to grazing intensity in semi-arid rangeland in western Iran

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### Abstract

Overgrazing is the main factor causing rangeland degradation in Iran. The main objectives of this study were to investigate the influence of grazing intensity on the palatability of the herbage and how bush encroachment is related to long-term grazing pressure. Herbage palatability was assessed as the weighted palatability composition of the vegetation considering palatability classes and the abundance of herbaceous and woody plant species. Grazing intensity as a management factor was estimated based on the livestock number of different grazing areas. To this end, 43 main plots were sampled in the rangeland areas of two catchments in the south-east of Kermanshah. High grazing intensity was negatively associated with biomass, species richness, herbaceous cover and palatability. Furthermore, the results indicated that encroachment of the rangeland by shrubs and invader species probably resulted from overgrazing. The results hint at overgrazing as a main factor for decreased palatability of the herbage in rangelands in Kermanshah.

Keywords: grazing intensity, rangeland management, palatability, vegetation attributes

#### Introduction

Rangeland is the main land use type of Iran. Due to heavy grazing pressure the productivity and availability of palatable plants have changed over the past few decades in rangelands of Iran (Mesdaghi, 1998). By selective grazing, the animals put more pressure on palatable than on unpalatable plants. When the numbers of animals exceed the carrying capacity of the rangeland, this may lead to encroachment of unpalatable species. In this study, we investigated the relationship between grazing pressure and palatability of herbage as well as woody plant encroachment in semi-arid rangeland in Kermanshah, Western Iran.

#### Materials and methods

The study was carried out in the rangeland areas of two catchments: the Kabodeh and the Merek catchment in Kermanshah province in western Iran. The study area was sub-divided into four separate communal grazing areas (GA). The livestock number of each grazing area (Livestock census, 2003) was used as a measure of grazing intensity (Table 1).

In grazing areas 1 to 4, the vegetation data were gathered from respectively 9, 9, 18 and 7 sites between May and July 2006 as follows. A total of 43 sites was selected according to a stratified double sampling design based on the distribution of range classes distinguished by visual interpretation of satellite images (Thompson, 2002). On each site, two perpendicular transects of 60 m each were set up. Per 2 m section of each transect, the occurring plant species were determined (species richness) and their expansion (per species) resulting in 30 data sets per transect.

To determine the palatability of the herbage, per site, the 20 dominant plant species were classified into palatability classes (PC; highly palatable (class I), medium palatable (class II), low palatable or unpalatable (class III), and poisonous species (class IV)) (Mesdaghi, 1998). For each plot, the cover of those of the 20 dominant species belonging to the same palatability class was accumulated. This was done for each of the four palatability classes, and it is called  $CPC1_{Plot}$  (the contribution of the palatability class 1 to the total cover per plot),  $CPC2_{Plot}$ ,  $CPC3_{Plot}$ , and  $CPC4_{Plot}$ . To allow the comparison between grazing areas, each  $CPC_{Plot}$  was divided by the total percentage cover of the 20 dominant species of the plot and then multiplied by 100. The result is called  $CPC1_{GA}$  (%),  $CPC2_{GA}$  (%),  $CPC3_{GA}$  (%), and  $CPC4_{GA}$  (%). It characterizes the relative contribution of each palatability class to the cover of the dominant plant species.

The results of the four GAs were compared using ANOVA followed by Tukey's HSD as a post-hoc test or the Kruskal–Wallis test followed by a post-hoc Mann-Whitney U-test for normally and non-normally distributed variables, respectively, using STATISTICA for Windows (Version 8.0) and SPSS (version 15).

#### **Results and discussion**

In this study, we tested the hypothesis that grazing intensity influences palatability of herbage and that bush encroachment is related to long-term grazing pressure. There are indications that grazing intensity was very important for vegetation composition and palatability. Thus, there was no difference (P < 0.05) between GA2, GA3 and GA4 in the important soil and topographic variables. Nevertheless, biomass and species richness of GA4 was higher (P < 0.05) than in GA2 and GA3 (Table 1). Furthermore, Table 1 indicates that the contribution of the medium palatable class II to the percentage cover (CPC2<sub>GA</sub>) was significantly higher in GA4 than in GA2 and GA3. As well, the CPC3<sub>GA</sub> (low or unpalatable species) in GA2 and GA3 was significantly higher than in GA4. The differences in biomass, species number and palatability of these grazing areas with the same environmental variables may result from different grazing intensity, which decreased from GA2 to GA4. These results are generally in line with previous findings that high grazing pressure reduces the standing biomass (Mwendera et al., 1997) and species richness (Wang et al., 2002). Hendricks et al. (2005) demonstrated that palatable grass species were reduced by heavy grazing and replaced with species of lower palatability.

The First GA (GA1) had a lower grazing pressure than the other GA. At the same time, the palatability of herbage was low in GA1. At first sight, this is astonishing, since we were postulating a deteriorating effect of high grazing pressures on palatability. However, farmers in the area suggested that the rangelands here had been degraded by heavy grazing over the last decades. More than 75% of livestock owners of the study areas believed that overgrazing (excessive number of livestock), and untimely grazing were the main reasons for the degradation (Faramarzi *et al.*, 2010). Badripour (2004) reported that often overgrazing resulted in a considerable extension of unpalatable, spiny, and poisonous species such as *Euphorbia* sp., *Phlomis* sp., and *Astragalus* sp. in Iran. In GA1, low palatable or unpalatable herbaceous species such as *Euphorbia* sp. (poisonous plant), *Gundelia tournefortii*, *Phlomis* sp., *Centaurea virgata* dominated. In addition, *Astragalus* sp. and *Daphne mucronata* dominated in the shrub layer of GA1. *Daphne mucronata* is a poisonous species for livestock and human beings (Durrani *et al.*, 2005). Therefore, livestock owners avoid areas with this shrub, leading to decreased grazing intensity and increased standing biomass.

	GA1	GA2	GA3	GA4
Total livestock dependent on rangeland (AU ha <sup>-1</sup> )*	0.57	3.75	2.88	0.82
CPC2 <sub>GA</sub>	$28.9\pm5.6^{\rm a}$	$30.0\pm16.7^{\rm a}$	$31.1 \pm 16.8^{\rm a}$	$41.2\pm6.6^{\rm b}$
CPC3 <sub>GA</sub>	$39.3\pm9.3^{\rm a}$	$42.3\pm12.8^{\rm b}$	$50.5 \pm 17.1^{b}$	$39.5\pm10.7^{\rm a}$
CPC4 <sub>GA</sub>	$19.5\pm8.1^{\mathrm{a}}$	$14.4 \pm 21.9^{\mathrm{a}}$	$1.6 \pm 2.4^{\text{b}}$	$6.2\pm3.3$ <sup>b</sup>
Biomass (g m <sup>-2</sup> )	$16.0\pm5.8^{\mathrm{a}}$	$5.8 \pm 1.6^{\rm b}$	$9.44 \pm 4.39^{b}$	$21.2\pm10.7^{\rm a}$
Herbaceous cover (%)	$28.2\pm9.6^{\rm ab}$	$25.2\pm5.8^{\rm a}$	$35.8\pm10.0^{\mathrm{b}}$	$37.5\pm8.7^{\mathrm{b}}$
Shrub cover (%)	$15.1 \pm 2.8^{a}$	$4.9\pm2.2^{\mathrm{b}}$	$3.0\pm2.5^{\text{b}}$	$2.0 \pm 1.7^{\mathrm{b}}$
Species richness (numeric)	$29.7\pm6.7^{\rm a}$	$28.1\pm5.8^{\rm a}$	$29.8\pm7.2^{\rm a}$	$40.3 \pm 4.8^{\mathrm{b}}$

Table 1. Means and standard deviations of the significant CPC1– $4_{GA}$  (%) define as well as vegetation attributes in the level of (P < 0.05)

\*In Iran, an animal unit (AU) is defined as one sheep or goat of approximately 40 kg (Mesdaghi, 1998). Different superscript letters indicate significant differences (P < 0.05) between GA.

#### Conclusions

The results of this study confirmed that high grazing intensity was negatively associated with palatability as well as vegetation attributes such as biomass, species richness, herbaceous cover, and shrub cover. Encroachment of the rangeland by shrubs and invader species probably resulted from overgrazing in the past. This shows the need for an adapted livestock management in the study areas.

#### References

Badripour H. (2004) Pasture/forage resource profiles of Iran. Rangeland and Watershed Management Organization (FRWO) – Ministry of Jihad-e-Agriculture, Iran.

Durrani M.J., Hussain F. and Saeed-ur-Rehman (2005) Ecological characteristics of plants of harboi rangeland, Kalat, Pakistan. *Tropical and Subtropical Botany* 13, 130–138.

Faramarzi M., Kesting S., Wrage N. and Isselstein J. (2010) Rangeland condition in relation to environmental variables, grazing intensity and livestock owners' perceptions in semi-arid rangeland in Western Iran. *The Rangeland Journal* 32, 367–377.

Hendricks H.H., Bond W.J., Midgley J. and Novellie P.A. (2005) Plant species richness and composition a long livestock grazing intensity gradients in a Namaqualand (South Africa) protected area. *Plant Ecology* 176, 19–33.

Livestock census (2003) Census of agricultural and livestock holdings (In Persian). Statistical Centre of Iran (SCI), Tehran.

Mesdaghi M. (1998) *Management of Iran's Rangelands* (In Persian), Imam Reza University, Mashhad, Iran. Mwendera E.J., Saleem M.A.M. and Woldu Z. (1997) Vegetation response to cattle grazing in the Ethiopian highlands. *Agriculture Ecosystems & Environment* 64, 43–51.

Thompson S.K. (2002) Sampling. Second Edition, J. Wiley & Sons, New York.

Wang Y.S., Shiyomi M., Tsuiki M., Tsutsumi M., Yu X.R. and Yi R.H. (2002) Spatial heterogeneity of vegetation under different grazing intensities in the Northwest Heilongjiang Steppe of China. *Agriculture Ecosystems & Environment* 90, 217–229.

# Grazing by horizon: what would be the limits to maintain maximum short-term herbage intake rate?

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## Abstract

Grazing animals continuously try to select the highest parts of the sward and, therefore the probability of grazing the lowest parts increases as the highest parts are gradually grazed. We hypothesized that as the lower horizons of the sward become accessible, animals would have their short-term herbage intake rate (STIR) reduced due to restrictions in sward structure. This study aimed to identify the remaining proportion of intact forage in the highest horizon of tropical grasslands (*Sorghum bicolor* cv. BR 501) from which the STIR of heifers is reduced. The treatments were proportions of depletion of the pre-grazing canopy height that maximizes the STIR (50 cm), as follows: 16, 33, 50, 67 and 84%. The experimental design was of randomized blocks with three replicates. The grazing tests lasted 45 min and the double-weighing technique was used to determine STIR. The canopy height was measured in pre, post-situation and every 15 min during depletion. The leaf lamina and stems+sheaths were distinguished in intact and grazed fractions. The STIR was reduced from 70–80% in the first horizon of the sward. From this point on, the proportion of intact leaf laminas started to reduce linearly.

Keywords: depletion, heifers, Sorghum bicolor, sward horizon

## Introduction

The grazing process corresponds to a sequence of bites where animals defoliate successive layers from the top to the bottom of the sward (Baumont *et al.*, 2004). The probability of grazing the lowest parts of the sward increases as the highest parts are gradually grazed (Un-gar *et al.*, 2001). In other words, animals preferably graze the leaves because this component of the sward provides a higher cost-benefit relation compared to stems. Therefore, a high short-term herbage intake rate is a function of the permanence time in the superior horizon of the sward, considering that this first horizon is characterized by a higher proportion of leaves (Baumont *et al.*, 2004). This study aimed to identify the remaining proportion of intact leaf laminas of the highest horizon of tropical grasslands (*Sorghum bicolor* cv. BR 501) from which the short-term herbage intake rate of heifers is reduced.

## Materials and methods

The experiment was carried out in an area of 4820 m<sup>2</sup> of *Sorghum bicolor* cv. BR 501, sown in February 2010. The treatments consisted of percentages of depletion: 16, 33, 50, 67 and 84%, equidistant between treatments. The experimental design was of randomised blocks with three replicates (n = 15). The blocking criterion was the time of day for the evaluations (morning or afternoon). The paddocks were scaled so that the percentage of depletion was achieved within approximately 45 min.

To determine the canopy height, a sward stick was used to measure 200 points per experimental unit both pre- and post-grazing. In addition, 50 measurements of canopy height were done every 15 min during depletion. During depletion, the leaves and stems + sheaths were distinguished in intact, grazed and damaged. These fractions were used to calculate the grazed and non-grazed areas of the experimental units. Three heifers were used (26±2 months old and 339±45.5 kg body weight), previously adapted to the experimental procedure. Although the animals were fasted from solids for five hours before the grazing tests, the fasting period was similar in all treatments, permitting valid comparisons. Fifteen grazing tests of approximately 45 min were conducted between 5 April and 15 April, and were always performed either during early morning or late afternoon.

Herbage mass was determined using three strata cut every 10 cm using a 0.153 m<sup>2</sup> square per paddock. The post-grazing herbage mass was sampled in 0.25 m<sup>2</sup> squares. Samples of post-grazing herbage mass were separated into leaf lamina, stem + sheaths and dead material and were then weighed. Next, they were dried at 55°C for at least 72 h to determine the dry matter (DM) content. The total herbage mass was determined as the sum of the mass of each component.

The short-term herbage intake rate (STIR) was determined using the double-weighing technique described by Penning and Hooper (1985). The STIR was corrected for the herbage DM content, estimated by cutting four samples from each experimental unit, two pre- and two post-grazing. The samples were collected until the canopy height reached the percentage of depletion for each treatment.

Data were analysed by a non-linear model  $(y_{ij} = a+b*log(x) + \in_{ij})$ , using the software JMP version 8 (SAS Institute Inc., Cary, NC, USA).

#### **Results and discussion**

Assuming a 'take-half' rule for mean bite depth (Laca, 1992; Ungar *et al.*, 2001), we expect that animals would graze the second horizon of the sward with approximately 50% of depletion, and from this depletion level on the STIR by animal would decrease. However, Fonseca *et al.* (submitted) observed a reduction in the STIR by heifers when 40% of the canopy height was grazed down. This difference can be attributed to the sward structure (Table 1), in which the proportion of intact leaf laminas remained constant until 40% of depletion.

Table 1. Actual depletion level (%), pre-grazing canopy height (cm), pre-grazing herbage mass (kg
DM ha-1), post-grazing herbage mass (kg DM ha-1), proportion of remaining leaf lamina (%) and
proportion of remaining stems+sheaths (%) of Sorghum cv. 501 swards under depletion levels grazed
by beef heifers (means; $n = 15$ )

		Deplet	ion lev	vel (%)		_			
-	16	33	50	67	84	Model	SE	$R^2$	Р
Actual deple- tion level	16.7	35.9	52.3	62.9	77.1	_	_	_	_
Pre-grazing canopy height	52.1	51.7	53.2	51.8	52.5	_	1.10	_	0.4405
Pre-grazing herbage mass	2706	2964	2530	2837	2673	_	576	_	0.9738
Post-grazing herb- age mass	1805	1379	1492	1328	840	y = 1968.47–1164.26x	228	0.57	0.0011
Proportion of re- maining leaf lamina	54.2	52.6	47.0	35.8	22.2	$y = 51.85 + 0.33(40 - x)^*$	10.5	0.50	0.0044
tion of remaining stems+sheaths	45.8	47.4	53.0	64.9	77.8	$y = 48.12 - 0.33(40 - x)^{\$}$	10.5	0.5	0.0044

SE = standard error,

P = significance level,

\* equation when x > 40, when x < 40 constant value of 51.85,

§ equation when x > 40, when x < 40 constant value of 48.12.

The STIR by heifer was reduced when the non-grazed area corresponded to 20–30% of the total area of the paddocks (Figure 1). We can affirm that the animals grazed by horizons and their STIR was reduced when they grazed the second horizon of sward, which has a higher proportion of stems+sheaths (Table 1). These results are in agreement with Ungar *et al.* (2001), which observed that heifers removed bites from the upper grazing horizon almost exclusively until approximately three-quarters of its surface area had been grazed. According to Baumont *et al.* (2004), when the uppermost horizon is dominant, the animals can easily graze on it and the preference for this horizon is higher. However, when the area occupied by the uppermost horizon decreased, the animals started to select the second horizon. This happens because the selection of the first horizon becomes tedious as the cost of searching for leaves outweighs the animal's benefit. This animal's disinterest in the first horizon begun exactly when the proportion of leaf laminas started to decrease and the stems+sheaths to increase linearly. In addition, these changes in the sward structure corresponded to the moment at which the STIR was drastically reduced.



#### Conclusions

The limit of transition between the first and the second grazing horizons to maintain maximum short-term herbage intake rate is of 70-80% of depletion of the sward's first horizon. From this point on, the sward structure is modified with a decrease in the proportion of intact leaf laminas and an increase in the proportion of stems + sheaths.

#### References

Baumont R., Cohen-Salmon D., Prache S. and Sauvant D. (2004) A mechanistic model of intake and grazing behaviour in sheep integrating sward architecture and animal decisions. *Animal Feed Science and Technology* 112, 5–28.

Fonseca L., Mezzalira J.C., Bremm C., Gonda H.L., Filho R.S.A. and Carvalho P.C.F. (submitted). Management targets for maximising the short-term herbage intake rate of cattle grazing in Sorghum bicolor.

Laca E.A., Ungar E.D., Seligman N.G. and Demment M.W. (1992) Effects of sward height and bulk density on bite dimensions of cattle grazing homogeneous swards. *Grass and Forage Science* 47, 91–102.

Penning P.D. and Hooper G.E.N. (1985) An evaluation of the use of short-term weight changes in grazing sheep for estimating herbage intake. *Grass and Forage Science* 40, 79–84.

Ungar E.D., Ravid N. and Bruckental I. (2001) Bite dimensions for cattle grazing herbage at low levels of depletion. *Grass and Forage Science* 56, 35–45.

# Comparison of dairy farming systems: indoor feeding versus pasture-based feeding

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## Abstract

The aim of this study was to compare indoor feeding (IF) to pasture-based feeding (PF) systems in dairy farming. From 2008 to 2010, we established two herds and kept them under the same conditions and within an equal agricultural area (AA). The IF herd consisted of 24 Swiss dairy cows, which were kept in a free-stall barn and fed a part-mixed ration (PMR) composed of maize silage, grass silage and a protein concentrate. The concentrate was given through a dispenser, according to the requirements of each individual cow. These cows produced 9,607 kg of energy-corrected milk (ECM) per lactation and 675 kg of milk fat and protein per standard lactation, having received 1,094 kg of concentrate. The PF herd consisted of 28 Swiss dairy cows, kept in a free-stall barn during wintertime and on a semi-continuous pasture. These cows produced 5,681 kg ECM per lactation and 435 kg milk fat and protein per standard lactation, having received 285 kg of concentrate. The calving interval and empty time of the PF cows were significantly shorter than those of the IF cows. The IF herd yielded 12,716 kg ECM (ha AA)<sup>-1</sup> y<sup>-1</sup>.

Keywords: dairy farming, indoor feeding, pasture, productivity, efficiency

## Introduction

Swiss farmers have been challenged to increase efficiency (FOAG, 2011). As such, the pasture-based production system in Switzerland has recently been intensively investigated (Schori and Münger, 2010; Thomet *et al.*, 2010). In North Carolina (USA), Vibart *et al.* (2008) showed that increasing intake of total mixed ration in an indoor feeding system results in higher milk and milk protein yields as well as in higher feed efficiency, whereas Dillon *et al.* (2005) suggest that pasture-based systems of milk production decrease unit production costs through lower feed cost and increased efficiency. However, would the advantages observed in North Carolina also apply to pre-alpine grassland conditions? From the perspective of productivity and efficiency in a pre-alpine Central European grassland region, we compared two herds: an indoor feeding herd and a pasture-based feeding herd. These herds had equal AAs on the same farm and received a specified feed from the available area.

## Materials and methods

The experiment was performed from 2008 to 2010 on a mixed farm belonging to the Vocational Education and Training Centre for Nature and Nutrition in Hohenrain, Central Switzerland (47°11'06.17" N, 8°19'08.03" E). The farm is located 620 m a.s.l., with an annual precipitation of 1,171 ( $\pm$ 131.0) mm and a mean air temperature of 9.4 ( $\pm$ 0.5) °C. We established two dairy herds: an IF herd and a PF herd. The average total AAs were 15.8 ( $\pm$ 0.37) ha for the IF

herd and 15.7 ( $\pm 0.70$ ) ha for the PF herd. On average, the IF herd was assigned 0.9 ( $\pm 0.40$ ) ha of pasture and hay area, and the PF herd was assigned  $13.7 (\pm 0.58)$  ha. In addition, the IF herd was assigned 6.8 ( $\pm 0.10$ ) ha of grass silage area and 2.9 ( $\pm 0.23$ ) ha of maize silage area. The share of ecological compensating area was 0.9 ha for both production systems. The IF herd and the PF herd were assigned 1.4 ( $\pm 0.32$ ) ha and 0.9 ( $\pm 0.24$ ) ha of maize and wheat meal area, as well as 2.9 ( $\pm 0.28$ ) ha and 0.16 ( $\pm 0.28$ ) ha of soybean cake and maize gluten feed area, respectively. The IF herd area was fertilised by  $177 (\pm 9.5)$  kg N ha<sup>-1</sup>; the PF herd area was fertilised by  $166 (\pm 2.2)$  kg N ha<sup>-1</sup>. Grass and maize silage were stored as round and cubic bales. An allowance was made for protein concentrate to be acquired from outside the farm. The IF herd consisted of 13 (±0.2) Brown Swiss (BS) and 11 (±0.8) Swiss Holstein-Friesian cows, with an average lactation number of 3.3 ( $\pm 2.1$ ) and an average body weight of 698 ( $\pm 86.2$ ) kg. The PF herd consisted of 14 ( $\pm 0.1$ ) BS and 14 ( $\pm 0.6$ ) Swiss Fleckvieh cows, with an average lactation number of 2.7 ( $\pm$ 1.3) and an average body weight of 610 ( $\pm$ 79.6) kg. The perennial feed of the IF cows was a PMR consisting of maize and grass silage, supplemented with a protein concentrate. From a daily output of 27 kg milk, the IF cows were individually fed a compounded concentrate by a dispenser, according to their individual requirements. At the onset of lactation (January to March), the PF cows were fed air-ventilated hay ad libitum and a maximum of 4 kg of concentrate  $cow^{-1} dav^{-1}$  in the barn. During the vegetation period, the PF herd grazed on a semi-continuous pasture subdivided into four paddocks.

Statistical analysis was performed with NCSS software (2004). We used an equal-variance t-test or Aspin-Welch unequal-variance test for normally distributed data and a Wilcoxon ranksum test for non-normally distributed data.

Limitation: The use of one herd per treatment did not allow any statistical comparison of variables related to area. In addition, the two herds were partially composed of different breeds; but their respective composition reflects the current use of such cow types in the corresponding production systems of the pre-alpine grassland region.

#### **Results and discussion**

The annual average yield of the hay pasture was 14.2 (±0.49); yield of the grass silage was 13.8 (±0.80); and yield of the maize silage was 17.7 (±1.92) t dry matter ha<sup>-1</sup>. On average, the full-time grazing period lasted 179 (±11.8) days, and the total grazing period was 242 (±2.4) days. The average IF stocking rate of 2.1 (±0.06) cows ha<sup>-1</sup> main forage area (MFA) was slightly higher than the PF stocking rate of 1.9 (±0.10) cows ha<sup>-1</sup> MFA. Table 1 shows that the crude protein value in pasture grass and hay stands out.

East 2008 2010	DM		DM	CP i	CP in DM		NDF in DM		ADF in DM		in DM
reeu 2006–2010	n	(g kg <sup>-1</sup> )		$(g kg^{-1})$		$(g kg^{-1})$		(g ]	kg <sup>-1</sup> )	$(MJ kg^{-1})$	
Grass silage	48	449	(82.3)	165	(29.2)	448	(47.1)	307	(25.4)	6.1	(0.4)
Maize silage	3	369	(20.4)	85	(5.0)	363	(31.4)	215	(20.7)	7.3	(0.2)
Pasture	51	159	(27.0)	260	(27.0)	379	(41.1)	242	(25.6)	6.3	(0.4)
Hay	5	873	(15.9)	161	(28.8)	427	(52.3)	290	(24.5)	6.1	(0.4)
Protein concentrate <sup>2</sup>	1	890		584		92		48		7.8	
Protein concentrate <sup>3</sup>	1	890		697		110		68		8.5	
Energy concentrate	1	890		117		99		38		8.1	
Compound feed	1	890		198		143		57		8.5	

Table 1. Chemical composition. Energy content of the feed (mean and SD)

<sup>1</sup>Net energy content for lactation according to Dairy One. Ithaca, New York, <sup>2</sup>2008–09, <sup>3</sup>2010.

According to the results of Vibart *et al.* (2008) we found that the milk performance and feed efficiency were higher in IF with PMR than in PF (Tables 2 and 3). The IF cows produced 241 kg

more milk fat and protein per lactation than the PF cows. This result is different from the findings of Fontaneli *et al.* (2005). The empty time of the IF cows lasted 43% longer. The AA productivity of the IF herd was 23% higher (Table 3). Interestingly, the PF herd produced a higher amount of ECM (ha AA)<sup>-1</sup> from the basic ration. This finding is primarily due to a lower concentrate supplementation. Values of feed conversion efficiency, i.e. kg ECM (kg BW<sup>0.75</sup>)<sup>-1</sup>, and of feed efficiency of the PF herd matches the results of Thomet *et al.* (2010) and Schori and Münger (2010).

Table 2. Milk performance and fertility of the dairy cows (mean and SD)

2008–2010	Ι	ndoor f	eeding	Past	ure-base	P-value <sup>1</sup>	
ECM from full lactation (kg)	67	9,607	(2,304.2)	88	5,681	(1,233.3)	**
ECM from standard lactation (kg)	62	8,900	(1,583.2)	67	6,074	(1,078.4)	**
Milk fat from standard lactation (g kg <sup>-1</sup> )	62	41	(2.7)	67	38	(3.7)	**
Milk protein from standard lactat. (g kg <sup>-1</sup> )	62	35	(1.9)	67	34	(1.5)	**
Milk fat from standard lactation (kg)	62	364	(68.9)	67	228	(42.6)	**
Milk protein from standard lactat. (kg)	62	311	(48.4)	62	207	(33.1)	**
Calving interval (days)	55	405	(58.9)	74	374	(30.1)	**
Empty time (days)	57	121	(57.5)	75	85	(29.6)	**

 $^{1}P < 0.01.$ 

Table 3. Productivity and efficiency (mean and SD)

2008–2010		Indoor fe	eding	Pasture-based feeding				
$ECM^{1}$ (ha AA) <sup>-1.2</sup> (kg)	3 <sup>3</sup>	1,2716	(201.3)	3	10,307	(616.5)		
ECM from basic ration (ha AA) <sup>-1</sup> (kg)	3	8,810	(254.2)	3	9,032	(781.0)		
NEL <sup>4</sup> in DM (MJ kg <sup>-1</sup> )	3	6.6	(0.02)	3	6.1	(0.10)		
ECM TDMI <sup>-1.5</sup> (kg kg <sup>-1</sup> ) i.e. feed efficiency	3	1.3	(0.04)	3	1.1	(0.03)		
ECM $(kg BW^{0.75})^{-1}(kg)$	3	61	(1.9)	3	47	(0.7)		
Concentrate air-dried cow <sup>-1</sup> lactation <sup>-1</sup> (kg)	3	1,094	(149.6)	3	285	(26.3)		
Concentrate air-dried kg <sup>-1</sup> ECM <sup>-1</sup> (g)	3	131	(14.7)	3	54	(6.0)		

<sup>1</sup>Energy-corrected milk, <sup>2</sup>Agriculture Area (i.e. producing area), <sup>3</sup>n = measurement y<sup>-1</sup>, <sup>4</sup>Net energy for lactation, <sup>5</sup>Total dry matter intake per cow.

## Conclusions

Based on the forage yield in pre-alpine regions of Europe, IF dairy cows fed with PMR and supplements according to their individual requirements are able to produce about 9,000 kg ECM with above-average milk solids. The productivity and the efficiency of the IF herd were higher compared to the PF herd; but more than 18% of the producing area was allocated to the protein concentrate, which had to be purchased outside of the farm. PF dairy cows are able to have a milk performance of about 6,000 kg ECM with higher fertility data than IF cows. A farm with PF is almost self-sustaining.

## References

Dillon P., Roche J.R., Shalloo L. and Horan B. (2005) Optimising financial returns from grazing in temperate pastures. In Murphy, J.J: Utilisation of grazed grass in temperate animal systems. *XX<sup>th</sup> International Grassland Congress*, July 2005, Cork, Ireland, 131–147.

FOAG (2011) Swiss Federal Office for Agriculture. Agricultural Report 2011. CH-3003 Berne.

Fontaneli R.S., Sollenberger L.E., Littell R.C. and Staples C.R. (2005) Performance of lactating dairy cows managed on pasture-based or in freestall barn-feeding systems. *Journal of Dairy Science* 88, 1264–1276. NCSS, 2004. J. Hintze, 329 North 1000 East, Kaysville, Utah 8403, 7 USA.

Schori F. and Münger A. (2010) Grazing behaviour and intake of two Holstein cow types in a pasture-based production system. *Grassland Science in Europe* 15, 895–897.

Thomet P., Piccand V., Schori F., Troxler J., Wanner M. and Kunz P. (2010) Efficiency of Swiss and New Zealand dairy breeds under grazing conditions on Swiss dairy farms. *Grassland Science in Europe* 15, 1018–1020. Vibart R.E., Fellner V., Burns J.C., Huntington B. and Green J.T. (2008) Performance of lactating dairy cows fed varying levels of total mixed ration and pasture. *Journal of Dairy Research* 75, 471–480.

# The effect of winter diet on pre-partum weight gain and post-partum milk production of grazing primiparous animals

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## Abstract

Forage brassicas, such as kale, are considerably cheaper as a winter feed than grass silage and concentrates, which are 2 and 4 times more expensive than grazed grass, respectively. An experiment was undertaken to investigate the effect of offering four different winter diets on heifer bodyweight (BW) and body condition score (BCS) during the winter period. Subsequent milk production performance was monitored when all heifers were managed similarly post-partum on a pasture-based system. One hundred Holstein-Friesian replacement heifers were balanced on the basis of age  $(639\pm20.5)$ days), projected calving date (23 February  $\pm$  22.0 days), BW (459 $\pm$ 43.8 kg) and BCS (3.21±0.215) and randomly assigned to one of four feeding treatments. The four treatments were i) indoors offered grass silage only (SO), ii) indoors offered grass silage plus 2 kg concentrate per day for a 46-day period (SC), iii) outdoors offered 70% kale + 30% grass silage (70K), and iv) 100% kale diet (100K). The experiment commenced on 15 November and heifers remained on their treatments until a week before calving. Following the winter period, daily weight gain was similar for the SC and 70K treatments  $(1.10 \text{ kg heifer}^{-1} \text{ day}^{-1})$ , weight gain was lower for the SO treatment (0.96 kg heifer^{-1})  $day^{-1}$ ), the weight gain of the 100K heifers was significantly (P < 0.001) lower than all other treatments (0.78 kg heifer<sup>-1</sup> day<sup>-1</sup>). There was no difference in cumulative milk yield or milk solids yield between treatments. The results from this study indicate that over-winter diet does not affect subsequent milk production performance.

Keywords: over winter diet, primiparous, milk yield, kale

## Introduction

The cost of rearing replacement heifers contributes significantly to the overall expense of milk production (Heinrichs, 1993) and thus it is essential that they calve at the correct BW, by 22 to 24 months of age. Many studies have shown a positive relationship between BW at calving and first-lactation milk yield (Clark and Touchberry, 1962; Lin *et al.*, 1987). There is also evidence to suggest that diet offered to heifers during the pre-partum period affects weight gain (Foot *et al.*, 1963; Mäntysaari *et al.*, 1999). Forage brassicas, such as kale (*Brassica oleracea*), are considerably cheaper as a winter feed than grass silage and concentrates, which are 2 and 4 times more expensive than grazed grass, respectively (Kavanagh *et al.*, 2008). Forage kale, offered to weanling heifers over the winter period, has previously been shown to be comparable, in terms of over-winter weight gain, to grass silage and concentrate (Kennedy *et al.*, 2011). However, there is limited information available on the effect of forage kale diets offered to nulliparous replacement dairy animals. The objectives of this study were to i) investigate the effect of winter diet on pre-partum weight gain of nulliparous replacement dairy animals. The objectives of this study were to i) investigate the effect of winter diet on pre-partum feeding treatment on post-partum milk production performance.

### Materials and methods

One hundred Holstein-Friesian replacement dairy heifers were balanced on the basis of age  $(639\pm20.5 \text{ days})$ , projected calving date (23 February  $\pm$  22.0 days), BW ( $459\pm43.8 \text{ kg}$ ) and BCS ( $3.21\pm0.215$ ) and randomly assigned to one of four feeding treatments. The treatments were i) indoors offered a silage only diet for the duration of the experiment (SO), ii) indoors offered silage and 2 kg concentrate for 46 days followed by a silage only diet – a total of 92 kg fresh weight of concentrate was offered to these animals (SC), iii) outdoors grazing forage kale in conjunction with grass silage bales at an inclusion rate of 30% in the diet (70K), and iv) outdoors grazing of a 100% forage kale diet (100K). The over winter experimental feeding treatments were imposed from 8 November 2010 until calving. The milk production data reported is from the first 29 weeks of lactation.

Prior to the commencement of the experiment all animals received an Allsure<sup>®</sup> bolus to provide iodine, selenium, copper and cobalt supplementation. The 100K-treatment animals were offered straw for the first week of the study to adjust them to the 100% kale diet. It was intended to offer no further fibre source after the first week to the 100K animals; how-ever, due to continuous frost 10 bales of silage were offered 6 weeks into the experiment for a duration of one-week.

All animals were offered fresh feed daily. The indoor animals were fed using a Griffith Elder electronic feeding system. The outdoor animals were offered a fresh allocation of kale each morning by moving a temporary electric fence. All treatments were grouped individually. During the experimental period all animals were weighed weekly and condition scored every three weeks. Post-parturition cows were turned out to grass directly and offered 4 kg DM concentrate for the first 40 days of lactation – all animals received the same concentrate input. Milk yield was recorded daily, while milk composition and BW were measured weekly. Body condition score was measured every three weeks. All data were analysed using PROC MIXED in SAS. Animal was used as the experimental unit. Pre-experimental values were used as a co-variate in the model.

#### **Results and discussion**

The concentrate fed during the winter period was 0.33 barley, 0.33 citrus pulp and 0.33 distillers grains. The DMD of the silage was 73 (±3.7)%, dry matter (DM) was 29.6 (±3.49)% and crude protein (CP) was 12.0 (±2.63)%. Following the winter period daily weight gain was similar for the SC and 70K treatments (1.10 kg heifer<sup>-1</sup> day<sup>-1</sup>), weight gain was lower for the SO treatment (0.96 kg heifer<sup>-1</sup> day<sup>-1</sup>), the weight gain of the 100K heifers was significantly (P < 0.001) lower than all other treatments (0.78 kg heifer<sup>-1</sup> day<sup>-1</sup>). Profile of heifer weight gain during the over-winter period can be seen in Figure 1. Pre-partum BW was greatest (P < 0.001) for the SC animals (555 kg) followed by the SO animals (535 kg). There was no significant difference between the 70K and 100K animals (518 kg) but they were significantly lighter than the indoor wintered herds. At the end of the winter feeding period BCS was highest (P < 0.001) for the SO and SC animals (3.47), the BCS of the 70K animals (3.25) was significantly lower than the indoor wintered animals yet higher than that of the 100K animals (3.09).

There was no significant difference between treatments in terms of cumulative milk yield or milk solids yield (3656 and 273 kg, respectively). There was no difference between treatments in average lactation fat, protein and lactose concentration (4.10, 3.38 and 4.70%, respectively). Average BW throughout the first 29 weeks of lactation was also similar between treatments (439 kg). Average BCS of animals from the 100K treatment was



Figure 1. Profile of heifer weight during the winter feeding period (100K – outdoors offered 100% kale diet; 70K – outdoors offered 70% kale diet and 30% grass silage; SC – indoors offered grass silage and 2 kg concentrate for 46 days; SO – indoors offered silage only)

lower (P < 0.001; 2.86) than that of the SC and SO animals (3.00) but was not different to the 70K animals (2.93). There was no difference in average BCS between the SC, SO and 70K treatments (2.98).

#### Conclusions

This study has shown that offering forage kale to nulliparous replacement dairy animals during the winter period results in pre-partum weight gain similar to that achieved by heifers housed indoors and offered grass silage and a total of 92 kg concentrate. Offering a silage-based diet indoors increased pre-partum BCS. However, the differences in pre-partum BW and BCS were not reflected in milk production differences as cumulative milk yield and milk solids yield were similar between all treatments. This study suggests that forage crops can be used as a viable alternative to grass silage-based feeding treatments over the winter feeding period.

#### References

Clark R.D. and Touchberry R.W. (1962) Effect of body weight and age at calving on milk production in Holstein cattle. *Journal of Dairy Science* 45, 1500–1510.

Foot A.S., Line C. and Rowland S.J. (1963) The effect of pre-partum feeding of heifers on milk composition. *Journal of Dairy Research* 30, 403-409.

Heinrichs A.J. (1993) Raising dairy replacements to meet the needs of the 21<sup>st</sup> Century. *Journal of Dairy Science* 76, 3179–3187.

Kavanagh S., Patton J. and Murphy J. (2008) Feed Costs 2008. In: Proceedings Teagasc National Liquid Milk Conference 2008. Dublin, pp. 10-21.

Kennedy E., Coughlan F., Fitzgerald S. and Murphy J.P. (2011) Effects of diet during the first winter on replacement heifer weight gain and body condition score. Proceedings of the Agricultural Research Forum 2011, Tullamore, p. 41.

Lin C.Y., Lee A.J., McAllister A.J., Batra T.R., Roy G.L., Vesely J.A., Wauthy J.M. and Winter K.A. (1987) Intercorrelations among milk production traits and body and udder measurements in Holstein heifers. *Journal of Dairy Science* 70, 2385–2393.

Mäntysaari P., Ingvartsen K.L. and Toivonen V. (1999) Feeding intensity of pregnant heifers: Effect of feeding intensity during gestation on performance and plasma parameters of primiparous Ayrshire cows. *Livestock Production Science* 62, 29–41.

# Adaptations of Type IV functional response for estimating short-term intake rate by grazing herbivores

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## Abstract

We evaluated and compared adaptative variations of Type IV functional response models – characterized by a quadratic model – for estimating short-term intake rate (STIR) by grazing cattle. Two experiments were carried out in *Cynodon dactylon* and *Avena strigosa* with swards representing different levels of canopy heights (10, 15, 20, 25, 30 and 35 for *Cynodon* and 15, 20, 25, 30, 35, 40, 45 and 50 cm for *Avena*). The STIR by animals was assessed from changes in pre- and post-grazing live weight with a correction for insensible weight losses, according to the double weighing technique. We hypothesized that the quadratic equation could not be the best model for estimating the maximum point of STIR. The JMP nonlinear models were used to fit five different models: regressions with breakpoint or plateau. The best model was selected by the smaller value of AICc. For *Cynodon* the best model was the double quadratic and for *Avena* the best alternative model was the double quadratic with plateau broken line. We argue that Type IV functional response can be adapted to explain better the response of STIR to different canopy heights.

Keywords: AICc value, foraging mechanisms, model likelihoods, model selection

## Introduction

The relationship between herbage intake rate and the availability of food items is described by a functional response (Holling, 1959) for which the shape of this functional response has been subject to numerous investigations. A proper understanding of factors that shape the functional response is thus of crucial importance in comprehending the dynamics and functioning of this response. For grazing herbivores, the short-term intake rate (STIR) has been found to increase asymptotically with grass biomass or canopy height as a Type II functional response (e.g. Delagarde et al., 2011). Countless studies (e.g. Casasús et al., 2004), however, have observed quadratic functional response to STIR (Type IV). This negative relationship between grass biomass and forage quality forces many grazers to trade-off between forage quality and herbage intake rate, with the highest nutrient and energy intake at intermediate sward biomass (Fryxell et al., 2004), leading to a dome-shaped relationship between grass biomass and nutrient or energy intake. Although the dome-shaped relationship has been the most commonly observed, we still need evolve in choosing the best model for responses in terms of plant-animal relationships. This study aims to assess distinct functional response models and evaluate alternatives to the quadratic model for estimating short-term intake rate by cattle.

#### Materials and methods

The study was based on two experiments. Experiment 1 was conducted between January and February of 2011 in an area of 13000 m<sup>2</sup> (25°45'00" S, 53°03'25" W) of *Cynodon dac-tylon* cv. Tifton 85, in place since 2008. The treatments consisted of six canopy heights: 10,

15, 20, 25, 30 and 35 cm. Six Jersey heifers (initial age of 20±2 months and average body weight of 318±13 kg) were used. Experiment 2 was conducted between July and September of 2011 in an area of 26000 m<sup>2</sup> (30°05'27" S, 51°40'18" W) of Avena strigosa cv. IAPAR 61, implanted in May of 2011 by 80 kg ha<sup>-1</sup> of seeds. The treatments consisted of eight canopy heights: 15, 20, 25, 30, 35, 40, 45 and 50 cm. Three crossbred heifers (Angus × Brahman), with initial age of  $45\pm2$  months and average body weight of  $349\pm20$  kg were used. The experimental design was randomized blocks with four replicates in both experiments. The area of the experimental units was approximately 500 m<sup>2</sup>. The canopy height was estimated by 200 pre- and 200 post-grazing measurements using a sward stick. The STIR by animals was assessed from changes in pre- and post-grazing live weight with a correction for insensible weight losses, according to the double weighing technique (Penning and Hooper, 1985). The STIR, expressed in g DM min<sup>-1</sup>, was calculated from the fresh weight of herbage and the DM content of six herbage samples by experimental unit, representative of that consumed by animals (superior half of sward), at before, mid and after the grazing period of 45 min. We used likelihood-based methods and information theoretics (Akaike corrected-Information Criterion, AICc) to quantify the strength of evidence for alternative models and to estimate their parameters. Maximum-likelihood estimates of model parameters, confidence intervals on model parameters, and AICc values were obtained by nonlinear fitting of model predictions using JMP software (version 8; SAS Institute Inc., Cary, NC, USA). The best

#### **Results and discussion**

The AICc provides an objective way of determining which model among a set of models is most efficient. It is rigorous, founded on solid statistical principles (i.e. maximum likelihood) (Burnham *et al.*, 2011). The results of AICc value (Table 1) showed that the double quadratic was the best model to describe the response of STIR by animals grazing distinct canopy heights in *Cynodon dactylon*. For *Avena strigosa*, in addition to the quadratic regression model (Type IV functional response), the double quadratic with plateau broken line model was the best alternative model and offered the best approximation of the data of STIR by animals grazing different sward structures.

models were selected by the smaller value of AICc (Akaike, 1974).

Estimated values by the best alternative models revealed that the quadratic model overestimated the maximum points of canopy height – in which the STIR is maximized – in 2.5 and up to 5 cm for *Cynodon* and *Avena*, respectively. It is important to highlight that the best model selected for Cynodon does not present a plateau, whereas the best alternative model for Avena presents a 4 cm plateau. This difference between the models indicates that the sward structure that maximizes the STIR in Cynodon is rapidly lost. On the other hand, the optimal sward structure in Avena could be maintained for more time, providing higher range for sward management to maximum STIR. Since management strategies aim to maximize energy intake rate over short time scales, the knowledge of the potential sward structures in relation to STIR is primordial. In the long-term, low differences in canopy height can be determinant to maintain a sward structure with higher leaf lamina/stem+ leaf ratio. Therefore, the selection of the best prediction model is important, considering that the suggested models proposed sward management targets with lower canopy heights than those estimated by the quadratic model. In addition, it is important to highlight that the suggested models provide independent intensities of increase and decrease of the STIR (equation coefficients) due to canopy height.

		Cyr	10aon e	actylon cv	. 85			
	-	Model	$\mathbb{R}^2$	P value	SE	AICc <sup>1</sup>	Max. STIR <sup>2</sup>	CH (cm) <sup>3</sup>
$\overline{\frown}$	Quadratic	$y = -\ 8.927 +\ 4.565 x\ -0.109 x^2$	0.70	< 0.0001	3.5	130.27	38.2	21.5
$\wedge$	Quadratic broken line	$\begin{array}{l} y1{=}~39.65{+}~({-}0.174)~*~(R-x)^2\\ y2{=}~39.65{+}~({-}0.88)~*~(x-R) \end{array}$	0.51	< 0.0001	6.2	148.44	39.76	20
$\frown$	Two breakpoints with plateau	y1=36.65 + (-0.97) * (R1 - x) y2=36.65 + 2.55 * (R2 - x)	0.10	0.329	6.1	154.87	36.65	20-30
$\cap$	Double quadratic with plateau broken line	$y1=37.54+(-0,14) * (R-x)^2$ $y2=37.54+(-0,07) * (x-R)^2$	0.67	< 0.0001	3.7	132.32	38.0	19–21
$\wedge$	Quadratic-plateau- linear broken line	$\begin{array}{l} y1{=}39.09 + (-0.17)^*(R1{-}x)^2 \\ y2{=}39.09 + (-1.01)^*(x{-}R2) \end{array}$	0.75	< 0.0001	3.2	148.79	39.0	19–21
$\wedge$	Double quadratic	$ \begin{array}{l} y1{=}38.0+(-0.12)^{*}\;(R-x)^{2}\\ y2{=}\;(-0.06)\;^{*}\;(x{-}R)^{2} \end{array} $	0.71	< 0.0001	3.5	129.73	37.9	19
		Aven	a strige	sa cv. IAPA	AR 61			
$\overline{\frown}$	Quadratic	$y = -11.59 + \ 3.751 x - 0.055 x^2$	0.81	< 0.0001	3.4	125.66	52.4	34
$\bigwedge$	Quadratic broken line	$ \begin{array}{l} y1 = 52.53 + (-0.10) * (R-x)^2 \\ y2 = 52.53 + (-0.55) * (x-R) \end{array} $	0.67	< 0.0001	4.4	135.18	52.5	30
	Two breakpoints with plateau	$\begin{array}{l} y1 = 52.12 + (-1.50) * (R1 - x) \\ y2 = 52,12 + 1.06 * (R2 - x) \end{array}$	0.45	0.003	5.7	146.06	52.1	28–38
$\bigcirc$	Double quadratic with plateau broken line	$\begin{array}{l} y1{=}51.04{+}\;({-}0.10)\;{*}\;(R{-}\;x)^2\\ y2{=}51.04{+}\;({-}0.04)\;{*}\;(x-R)^2 \end{array}$	0.74	< 0.0001	3.9	129.50	51.0	29–33
$\wedge$	Quadratic-plateau- linear broken line	$\begin{array}{l} y1{=}51.70 + (-0.11)^*(R1{-}x)^2 \\ y2{=}51.70 + (-0.64)^*(x{-}R2) \end{array}$	0.66	< 0.0001	4.5	135.72	51.7	29–33
$\wedge$	Double quadratic	$y_{1}=51.6 + (-0.09)^{*} (R - x)^{2}$ $y_{2}=51.6 + (-0.03)^{*} (x - R)^{2}$	0.68	< 0.0001	4.4	129.73	51.6	30

Table 1. Models for predicting short-term intake rate by animals (STIR) in relation to canopy heights (CH)

<sup>1</sup>Akaike corrected-Information Criterion; the smaller value represents the best model (Akaike, 1974), <sup>2</sup> maximum value of STIR, estimated by the model, <sup>3</sup> values of CH in which the STIR is maximized, estimated by the model.

#### Conclusions

The best alternative models of Type IV functional response for estimating short-term intake rate by herbivores grazing *Cynodon* and *Avena* are the double quadratic and the double quadratic with plateau broken line, respectively. By the suggested models, the short-term intake rate declines in tall swards at a smaller intensity than the decline observed in smaller canopy heights.

#### References

Akaike H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19, 716–723.

Burnham K.P., Anderson D.R. and Huyvaert K.P. (2011) AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology* 65, 23–35.

Casasús I., Sanza A., Villalba D., Ferrera R. and Revilla R. (2004) Intake capacity of two breeds of suckler cattle of different milk yield potential and validation of prediction models. *Livestock Production Science* 89, 195–207.

Delagarde R., Valk H. and Mayne C.S. (2011) GrazeIn: a model of herbage intake and Milk production for grazing dairy cows. 3. Simulations and external validation of the model. *Grass and Forage Science* 66, 61–77. Fryxell J.M., Wilmshurst J.F. and Sinclair A.R.E. (2004) Predictive models of movement by Serengeti grazers. *Ecology* 85, 2429–2435.

Holling C.S. (1959) The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. *Canadian Entomologist* 91, 293–320.

Penning P.D. and Hooper G.E.N. (1985) An evaluation of the use of short-term weight changes in grazing sheep for estimating herbage intake. *Grass and Forage Science* 40, 79–84.

# Daily forage production in pastures of Elephant grass (*Pennisetum purpureum* Schum.) managed with different post-grazing heights

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## Abstract

The daily forage production was evaluated on swards of Elephant grass cv. Napier (*Pennisetum purpureum* Schum.) subjected to rotational stocking management characterized by three post-grazing heights (30, 50 and 70 cm) and grazings carried out at the 95% canopy light interception condition from February to May in 2009 (experiment 1) and from December 2009 to May 2010 (experiment 2). The experimental design was a randomized complete block with three replications. Swards managed with the 30 cm post-grazing height presented lower daily forage production in relation to those managed with the post-grazing heights of 50 and 70 cm [80 vs. 183 and 179 kg ha<sup>-1</sup> day<sup>-1</sup> of DM (experiment 1)] and [57 vs. 116 and 145 kg ha<sup>-1</sup> day<sup>-1</sup> of DM (experiment 2)]. Swards managed with the 70 cm post-grazing height presented larger stem and dead material accumulation (around 27 and 30%, respectively) than those managed at 50 cm. Elephant grass cv. Napier should be managed with a 50 cm post-grazing height.

Keywords: grassland management, light interception, Pennisetum purpureum

## Introduction

Recent studies with various tropical grasses revealed a fairly consistent pattern of forage accumulation according to which grass reduces leaf accumulation, enhances stem elongation and leaf senescence rates after the canopy reached 95% of light interception (LI) (Da Silva and Nascimento Júnior, 2007). Thus, the timing of regrowth interruption seems to be well defined. However, the defoliation severity in grazing systems needs to be adjusted to ensure a residual leaf area large enough to promote fast and efficient regrowth without compromising the plant's organic reserves and/or sward structure. In this context, knowledge regarding the forage accumulation process has proven to be of great importance in helping to define management goals. Thus, the aim of this study was to assess the daily forage production of Elephant grass (*Pennisetum purpureum* Schum.) cv. Napier subjected to three severities of rotational stocking management.

## Materials and methods

The study was conducted in an area cultivated with Elephant grass cv. Napier in Vicosa, MG, Brazil (20°45'S; 42°51'W; 651 m a.s.l.). The pastures of Elephant grass were established in December 2007 and were kept under rotational grazing until the beginning of the experimental period (February, 2009). The climate, according to Köppen, is Cwa subtropical, with well-defined dry (winter) and rainy seasons (summer). Mean annual temperature and rainfall are 19 °C and 1,340 mm, respectively. Soil is classified as Inceptic Hapludults, with clay-loam texture.

Because of early apical meristem lengthening in Elephant grass cv. Napier and significant aerial tillering after decapitation of apical meristems of basal tillers, two experiments were

conducted to access three post-grazing heights: severe (30 cm), representing a high level of forage utilization; intermediate (50 cm); and lenient (70 cm). In experiment 1, conducted from February to May 2009, animals began grazing when the canopy reached 95% LI regardless of the initial decapitation height of the apical meristems of the basal tillers during first grazing. In experiment 2, conducted from December 2009 to May 2010, first grazing was conducted when the meristems reached the specified post-grazing target height of 30, 50 or 70 cm, regardless of canopy LI. Subsequent grazing cycles were carried out consistently at 95% canopy LI. In both experiments treatments were assigned to experimental units (400 m<sup>2</sup> paddocks) according to a randomized complete block design with three replications. Swards were fertilized with 200 kg ha<sup>-1</sup> of nitrogen (N), applied in installments using ammonium sulphate in the post-grazing. Thus, 200 kg ha<sup>-1</sup> N was divided by the period of grass growth (January-March) to obtain the daily amount to be applied to each paddock. The applied amount of fertilizer was calculated by multiplying the daily amount by the rest period that occurred in each experimental unit.

The LI was monitored using a canopy analyser apparatus (LAI, 2000) at 20 points per experimental unit. In experiment 2, the height of the apical meristems of the basal tillers was used to determine the start of first grazing. Thus, 20 tillers were cut to ground level and then cut longitudinally to expose their apical meristems, the height of which was measured as the distance between the tiller base and the apical meristem apex.

Daily forage production was assessed in 12 tillers per experimental unit. These tillers were randomly marked (aerial and/or basal) at the onset of each regrowth period and were assessed twice a week by measuring the length of the leaf blades and stems (stem + leaf sheaths). During the assessment period, any aerial tillers that emerged from the marked tillers were incorporated into the monitored group and assessed similarly. These assessments enabled us to estimate the rates of leaf and stem elongation (cm tiller<sup>-1</sup> day<sup>-1</sup>) (Lemaire and Chapman, 1996). On the last day of each assessment period, each marked tiller was cut to ground level (basal tillers) and/or at its point of insertion (aerial tillers). Leaf blades and the stems from each tiller were dried in a forced-draught oven at 65°C for 72 h. After drying, the material was weighed, and the mass of each component divided by its respective length, generating a mass-length conversion factor that was used to transform all of the field readings from cm tiller<sup>-1</sup> day<sup>-1</sup> to mg tiller<sup>-1</sup> day<sup>-1</sup>. Final transformation to kg ha<sup>-1</sup> day<sup>-1</sup> for the dry forage mass (DM) was performed multiplying these values by the tiller population density. Tiller population density was determined by counting all live aerial and basal tillers within four existing  $0.25 \times 1.00$  m metallic frames per experimental unit. Thus, the sum of the daily leaf and stem production was used as the daily forage production total growth rate. Data were subjected to analysis of variance using the MIXED procedure of the SAS statistical package. Means were estimated by LSMEANS and compared using Student's *t*-test at a 5% significance level.

#### **Results and discussion**

In experiment 1, daily forage production was influenced by post-grazing height (P = 0.0007) and month of the year (P = 0.0021). Larger values were recorded on swards managed at 70 (183 kg ha<sup>-1</sup> day<sup>-1</sup> of DM) and 50 cm (179 kg ha<sup>-1</sup> day<sup>-1</sup> of DM) post-grazing height than on those managed at 30 cm (80 kg ha<sup>-1</sup> day<sup>-1</sup> of DM). In experiment 2, daily forage production was also influenced by post-grazing height (P = 0.0352) and month of the year (P < 0.0001). Again, larger values were recorded on swards managed at 70 (145 kg ha<sup>-1</sup> day<sup>-1</sup> of DM) and 50 cm (116 kg ha<sup>-1</sup> day<sup>-1</sup> of DM) post-grazing height than on those managed at 30 cm (57 kg ha<sup>-1</sup> day<sup>-1</sup> of DM).

Regardless of management strategy (experiment 1 or 2), swards managed with post-grazing height 30 cm showed the lowest daily forage production. The most severe grazing removes larger quantities of forage and results in increased decapitation of tillers. Under those conditions, initial regrowth is slow due to a smaller amount of leaves after grazing and the need to allocate photoassimilates for the differentiation and division of new cells to form new meristems and tillers. On the other hand, as all swards were grazed at 95% canopy LI the lower daily forage production recorded on swards managed with the post-grazing height of 30 cm suggests that grazing was too severe under those circumstances. Additionally, those swards had lower tiller density larger proportion of weeds than those managed at 50 and 70 cm, indicating that the 30 cm post-grazing height was not adequate for the Elephant gras cv. Napier.

Daily forage production did not differ between the post-grazing heights of 50 and 70 cm, indicating that both post-grazing heights could be used for Elephant grass cv. Napier. However, swards managed with the post-grazing height of 70 cm showed 27 and 30% larger accumulation of stem and dead material, respectively, relative to those managed at 50 cm. Such condition may affect herbage intake by grazing animals as stems correspond to a physical barrier to defoliation that reduces the ease of forage prehension, interfering negatively with grazing efficiency (Palhano *et al.*, 2007).

#### Conclusions

Elephant grass cv. Napier should be managed with a post-grazing height of 50 cm.

#### References

Da Silva S.C. and Nascimento Júnior D. (2007) Avanços na pesquisa com plantas forrageiras tropicais em pastagens: características morfofisiológicas e manejo do pastejo. *Brazilian Journal of Animal Science* 36, 121–138.

Lemaire G. and Chapman D. (1996) Tissue flows in grazed plant communities. In: Hodgson J. and Illius A.W. (eds) *The ecology and management of grazing systems*, CAB International, Wallingford, UK, pp. 3–36. Palhano A.L., Carvalho P.C.F., Dittrich J.R., Moraes A., Da Silva S.C. and Monteiro A.L.G. (2007) Características do processo de ingestão de forragem por novilhas holandesas em pastagens de capim-mombaça. *Brazilian Journal of Animal Science* 36, 1014–1021.

# Grazing-induced patches in the Inner Mongolia steppe as affected by grazing intensity and management system

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## Abstract

The selective grazing of sheep influences sward structure by inducing heterogeneous vegetation patches. Some areas are favoured by sheep, while others are permanently avoided. Ungrazed patches may provide good soil protection but also exhibit low forage quality owing to the large amounts of mature and fibrous plant biomass. In contrast, grazed patches typically exhibit a relatively high risk of erosion but also higher forage quality compared to ungrazed patches. Adequate management and grazing intensity are required.

The present study highlights the effect of grazing intensity (ungrazed up to very heavily grazed) and management system (Mixed System, annual alternation between grazing and haying; vs. Traditional System, continuous separation between grazing and hay-making area) on grazing patches at the small scale. In relation to the grazing intensity and grazing patches, maps of biomass distribution obtained by GIS illustrate lower heterogeneity at high grazing intensity compared to the moderately grazed plots. Moderate grazing intensity induces relatively heterogeneous sward conditions including overgrazed hotspots with an increased risk of degradation. As an effect of management systems the biomass maps show that patches are more pronounced in the Traditional System compared to the Mixed System. Moderate grazing intensity does not necessarily imply sustainability at any scale.

Keywords: grazing pattern, semiarid grassland, sheep grazing, vegetation maps

## Introduction

Grazing patches as an effect of selective sheep grazing are exposed to high grazing pressure. Such overgrazed patches exhibit increased risk of degradation, especially in semiarid grassland. The phenomenon of grazing patches is described in the literature (e.g. Vallentine, 2001). But there is not much known about the effect of grazing intensity and management system on grazing patches in fenced pastures, especially in the steppe grassland of Inner Mongolia. The present study focuses on grazing patches as affected by grazing intensity and management system. Here we present results using GIS software giving a geo-referenced view at the small scale biomass distribution.

## Materials and methods

The study site was located in semiarid, native grassland within the Xilin River catchment, Inner Mongolia Autonomous Region, P.R. China (43°38' N, 116°42' E). The investigated typical steppe ecosystem is characteristically dominated by the perennial rhizome grass *Leymus chinensis*, and the perennial bunchgrass *Stipa grandis*. The average temperature and precipitation in the region is 0.9°C and 329 mm (1982–2010). The grazing experiment was established in 2005, i.e. 4 years before the present measurements. In two field replicates, three different grazing intensities (ungrazed; moderately grazed (0.8±0.07 sheep ha<sup>-1</sup> year<sup>-1</sup>); and heavily grazed (1.9±0.08 sheep ha<sup>-1</sup> year<sup>-1</sup>)) and two different management systems were compared. In the Mixed System, annual alternation between grazing and hay making was applied, while in the Traditional System the same area was always used either only for grazing or hay making. Sheep were kept on the plots day and night from May-September during the 150 days of the growing season. The 2-ha sized plots were structured with a  $10 \times 10$  m grid to analyse the vertical and horizontal distribution of the vegetation. The grazing patches were assessed by height × density measurements at each grid point using a Rising-Plate meter (Grasstec). Height measurements are an appropriate tool to estimate aboveground standing biomass in the investigated area (Schönbach *et al.*, 2008). Georeferenced data were digitised and interpolated by using ArcGIS 9.2 software. The coefficient of variation (CV), calculated by biomass height of 200 grid points per plot, was used to compare heterogeneity of swards among treatments.

#### **Results and discussion**

Treatment plots showed differences in the vertical and horizontal aboveground biomass distribution. The relation between grazing intensity and biomass distribution revealed that heavy grazing intensity led to lower heterogeneity compared to the moderately grazed plots in the Traditional System (Figure 1). The maps illustrate overgrazed patches (i.e. areas of low vegetation cover) in moderately grazed plots in the Traditional System. More than one quarter of the data in the moderately grazed plot showed a height of biomass lower than 5 cm. Biomass heights between 0.6 cm and 2.5 cm were observed for 8.1% of the collected data at this plot. Sheep heavily grazing the same areas every year resulted in the same negative overgrazing effects as were found in the completely heavily grazed plots. Moderate grazing *per se* does not necessarily imply sustainability at any scale, as it depends strongly on the management system used.



Figure 1. Biomass distribution in relation to grazing intensity

The comparison of biomass distribution in management systems at moderate grazing intensity revealed more heterogeneity in the Traditional System than in the Mixed System (Figure 2). Our results suggest that sheep returned to the same areas every year in the Traditional System. The selective behaviour of sheep resulted in progressive differentiation of grazed and rejected areas over time. In these areas of different biomass height we found on a small scale the same typical effects as in completely heavily grazed or ungrazed plots. These overgrazed patches lead to a lower productivity. From the perspective of environmental protection the risk of erosion and degradation increases with a lower biomass height because of the low vegetation cover (Schönbach *et al.*, 2009).



Figure 2. Management System in relation to grazing pattern

An indicator for the variation of the biomass is the coefficient of variation (CV). The cv gives an idea of the heterogeneous distribution but is not related to the spatial distribution. The cv is higher in the Traditional System (mean CV 0.454) than in the Mixed System (mean CV 0.276). Annual alternation between hay-making and grazing reduced the heterogeneity in the Mixed System, with less pronounced heterogeneous grazing patches. These results suggest that the management system may be as important as grazing intensity for sustainable management of semi-arid grasslands.

#### Conclusions

The distribution of plant biomass was influenced by both grazing intensity and management system. Moderate grazing intensities induced relatively heterogeneous sward conditions, including overgrazed hotspots, particularly in the Traditional system. High grazing intensity led to more homogenous biomass distribution. In the moderately grazed intensities in the Traditional System the grazing patches were overgrazed because the sheep returned to the same areas every year. The Mixed System, with an annual shift between grazing and hay making, showed a more homogenous biomass distribution and grazing patches were less pronounced. These results suggest that management system may be as important as grazing intensity for sustainable management of semi-arid grasslands. Also, moderate grazing intensity with fixed management systems (only grazing or haymaking) does not imply in sustainability at any scale.

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#### References

Schönbach P., Wan H., Gierus M., Bai Y. and Taube F. (2008) Relationship between sward surface height and agronomic traits in the typical steppe of Inner Mongolia. In: International Grassland and International Rangelands Congress p. 1160. Hohote, China: Supplementary 2008 XXI International Grassland and VIII International Rangelands Congress Proceedings.

Schönbach P., Wan H., Schiborra A., Gierus M., Bai Y., Müller K., Glindemann T., Wang C., Susenbeth A. and Taube F. (2009) Short-term management and stocking rate effects of grazing sheep on herbage quality and productivity of Inner Mongolia steppe. *Crop and Pasture Science* 60, 963–974.

Vallentine J.F. (2001) Grazing Management. Second Edition, California, USA: Academic Press, 659 pp.

# Long-term effects of all-year beef cattle grazing on characteristics of soil chemistry and vegetation

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## Abstract

Recent scientific approaches to optimize all-year cattle grazing under conditions in Central Europe are generally aimed to solve specific problems of winter grazing. The systemorientated validation of scientific data across different types of farms with long experience in professional all-year outdoor grazing has not been realized yet. Botanical surveys and soil samples were taken and analysed in farms managed in full-time or part-time under different environmental conditions in western Germany. The criteria for choosing the farms in this experiment were: year-round grazing since many years (up to 40 years), no application of mineral fertilizer and herbicides, and no repair-seeding. Despite the complete absence of fertilization and under conditions of high amounts of annual rainfall, the concentrations of potassium in soil and, in some cases of phosphorus, were considerably high. Negative effects on the botanical composition affecting ecological stability, forage value and biodiversity were negligible. Botanical indicators for soil compaction are limited to spots next to feeding places and favoured places to lie. Summarizing all results, all-year beef cattle grazing is a sustainable and interesting land-use option for less productive grassland even under cold climatic conditions (average of >100 days of freezing, mean temperature  $<8^{\circ}$ C) in lowmountain regions in Central Europe.

Keywords: year-round grazing, long-term effects, botanical biodiversity, forage value

## Introduction

Compared with regions with a more oceanic climate, year-round grazing is not common in Central Europe. Scientific knowledge on the effects of this grazing system in regions with harsh winter conditions is limited. More recent work considers specific problems of winter grazing such as the availability, quality and ensilability of forage during winter (Opitz v. Boberfeld and Wöhler, 2002; Wolf and Opitz v. Boberfeld, 2003; Laser, 2005; Opitz v. Boberfeld and Banzhaf, 2005) and specific environmental effects of winter-grazed pastures as nutrient accumulation in soil (Opitz v. Boberfeld *et al.*, 2005), soil compaction (Opitz v. Boberfeld *et al.*, 2007). This paper considers long-term effects of year-round grazing on floristic biodiversity, forage value, and soil pH value and nutrient content. For this purpose, pastures of farms practising such systems for many years in full-time or part-time management (= additional off-farm income) were examined.

## Materials and methods

Table 1 gives a short overview of the farms. All farmers in the study manage the grassland without herbicides, repair seeding and fertilization. Botanical surveys were taken according to Klapp-Stählin (Klapp, 1929) during mid May and early July in plots measuring  $100 \times 100$  m on every pasture of the farms. Soil samples were taken in a grid of  $200 \times 200$ 

m (=389 botanical surveys and 207 soil samples). Plant available phosphate and potassium were measured in an extract of calcium acetate lactate, and pH was determined using a glass electrode with 0.01 M CaCl<sub>2</sub> (Anonymous, 1991). The forage value was classified according to the scale of Klapp *et al.* (1953), in which –1 means toxic, 0 means worthless or uneatable and 8 is the highest forage value. Due to different farm sizes, different numbers of surveys, and uneven spread, all data sets were analysed using non-parametric statistics (Siegel, 2001). The figures show the specific means, minima and maxima to each farm and the upper and lower quartiles (.25 and .75 quartile).

form any ironmont _	full	-time	part-	time	
	А	В	С	D	А
since (year)	1965	1989	1992	1986	1983
m above sealevel	300	360	450	450	390
annual rainfall (mm)	790	960	1000	1000	938
average temperature (°C)	7.8	7.5	6.9	6.9	7.3
farm characteristics					
area (ha)	100	150	74	53	94
number of pastures	7	18	13	23	15
breed	Angus	Limousin	Aubrac	Salers	_
suckler cows	69	76	30	22	49
beef cattle	95	97	25	10	57
herds	4	3	2	1	3

Table 1. Overview on farms

#### **Results and discussion**



The soil of 20% of all studied pastures was hydromorphic. Acid Cambic Umbrisols were dominant with some Coluvic Regosols in the valleys. The soil pH value was lower in the part-time farms (Figure 1), probably because of a higher rate of area that was not owned by the farmer leading to lower expenditures for liming. Regarding the annual rainfall, zero fertilization, and the development of soil based on greywacke, one could have expected lower concentrations of potassium in soil (Figure 1). But the potassium level can, in most cases, be classified as 'optimum' according to the German assessment system. The plant communities on full-time farms were mainly Lolio-Cynosuretum with high proportions of Lolium perenne (average 23%) and Trifolium repens (average 5%). On parttime farms (mainly Festuco-Cynosuretum) the dominant grass was Festuca rubra at farm c (average 27%) or Agrostis capillaris at farm d (18%) accompanied by Trifolium repens and Trifolium pretense (average 2% each) which were the most important legumes. Indicator plants for slightly compacted and also moist soils were more frequent in the

Figure 1. Chemical traits of 207 soil samples in sum

*Festuco-Cynosuretum* communities than in others. Strong indicator plants for soil compaction were negligible in all pastures. Typical indicators for high nitrogen concentration in soils (*Cirsium arvense, Elymus repens, Rumex obtusifolius*) were found with low consistency in small proportions. The forage value of pastures on full-time farms on average is much higher than on part-time farms (Figure 2), whereas there was only a small difference concerning the biodiversity. The Shannon-index was high for both farm types.



Figure 2. Forage value scale and botanical biodiversity

#### Conclusions

Potential negative effects of year-round grazing on vegetation, soil, forage value and soil compaction in the long term can be assessed to be tolerable in most cases. Differences between full-time and part-time management are difficult to assess. High environmental variability in extensively managed grassland interferes with management effects.

#### References

Anonymus (1991) Methodenbuch 1. Die Untersuchung von Böden. 4th Ed., Verl. VDLUFA, Darmstadt. Klapp E. (1929) Thüringische Rhönhutungen. *Wiss. Arch. Landw. Arch. Pflanzenbau* 2, 704–786.

Klapp E., Boeker P., König F. and Stählin A. (1953) Wertzahlen der Grünlandpflanzen. *Das Grünland* 2, 38-40. Laser H. (2005) Pflanzenbauliche Ansätze zur Selenversorgung von Mutterkühen und Fleischrindern in Weidesystemen. Post-doctoral thesis. Gießen.

Opitz v. Boberfeld W. and Wöhler K. (2002) Forage quality of low input winter pastures under varying conditions in central Germany. *Grassland Science in Europe* 7, 222–223.

Opitz v. Boberfeld W., Sterzenbach M. and Laser H. (2005) Accumulations of N, P and K in soil in different systems of out door keeping during winter with cattle. *Austrian Journal of Agricultural Research* 56, 53–60. Opitz v. Boberfeld W. and Banzhaf K. (2006) The effects of sward management on mineral content of winter grazed herbage. *Journal of Agronomy and Crop Science* 192, 1–9.

Opitz v. Boberfeld W., Schlimbach G. and Schröder D. (2007) Zum Einfluss der Winterbeweidung auf bodenphysikalische Merkmale. Zeitschrift für Pflanzenbauwiss enschaften 11, 20–27.

Siegel S. (2001) Nichtparametrische statistische Methoden. 5th Ed., Fachbuchverl. Psychologie.

Wolf D. and Opitz v. Boberfeld W. (2003) Effects of nitrogen fertilization and date of utilization on the quality and yield of tall fescue in winter. *Journal of Agronomy and Crop Science* 189, 47–53.

# Practical application of sensor registered grazing-time and -activity for dairy cows

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## Abstract

A possibility for a new farm application to optimize grazing management focuses on estimating grazing time. In two experiments, activity data were collected using accelerometers attached to grazing animals. Grass intake was estimated by the method of energy balance. Grazing time was computed from acceleration data (GRacc). GRacc data showed a sensitivity of 74% and specificity of 82%, when compared to manual observations. GRacc was tested for correlation with grass intake: the best correlation (0.82) was observed for cows with restricted feeding in the barn, and strip grazing on grass with a height of 16 cm.

Keywords: accelerometer, feed intake, grazing, precision farming

## Introduction

Farmers who graze their cattle need information about pasture use and feed intake (Oudshoorn, 2009). Increasing herd size, higher milk yield, and lack of adjacent pasture can jeopardize sufficient and steady grass intake and cause farmers to stop pasturing cows. Technological developments have provided tools for automatic registration of animal behaviour (accelerometers, pitch and roll indicators, digital compass and global navigation satellite systems) that can be integrated with farm management software. Examples of this type of application are oestrus detection and animal location (e.g. Heatime<sup>®</sup>, CowDetect<sup>®</sup>). A new farm application could be developed with a focus on estimating pasture time, grazing time and grazing activity, in order to better manage feed resources. The objectives of these studies were to analyse if accelerometer sensors can be used to estimate grass intake; to determine if accelerometers that were tightly or loosely attached made a difference and if grass height or stocking rate, as a measure of herbage allowance (HA), influence the results.

## Materials and methods

One experiment (EX1) was conducted in 2009 for 2 weeks and one (EX2) in 2010 for 10 days, both in the autumn. Both experiments were conducted at the research centre Foulum, Denmark (56°N, 9°E). The cows were outside during daytime; at night they were fed known amounts of a total mixed ration (TMR); ad libitum in EX1 and restricted in EX2. Milk yield and composition were registered during the whole experiment on an individual basis. Grass intake was estimated daily by using the calculated energy requirement of the individual cow with respect to her milk yield and maintenance requirement. For each experiment, the estimated grass intake was compared to acceleration data for the corresponding days. The grass height was measured regularly using a C-Dax pasture meter, and the heights reported are averages of all days; dry matter content of grass was also determined.

EX1: Twenty cows grazed in a permanent grazing system from approx. 8:00 a.m. to 3:00 p.m., and their activity was registered throughout that period using two-axis accelerometers (MTS310 sensor board, one sample per second, range 0-2 g, connected by wireless sensor network) fitted tightly on the top of the neck. The cows were divided into two groups:

Low HA (n = 10): refers to a stocking density of 4.6 cows ha<sup>-1</sup>; High HA (n = 10) refers to a lower stocking density of 3.5 cows ha<sup>-1</sup>. Grass height was approx. 17 cm in both pastures (1.08 ha and 1.42 ha, respectively). Additionally, manual registration of grazing time (GR-man) was performed by a technician, systematically, at 10-minute intervals.

EX2: Ten cows grazed from approx. 7:00 a.m. to 14:30, and their activity was registered in that period using two three-axis accelerometers (ADXL345, one sample per second, range 0–2 g, data downloaded): one sensor was fitted firmly on the top of the neck (tight), and one was loosely fitted below the neck (loose). The cows were divided in two groups: H11 (n = 5) refers to grass height of 11 cm, in a permanent grazing system; H16 (n = 5) refers to a grass height of 16 cm in a rotational strip grazing system.

For each experiment, grazing time was computed from raw accelerometer data (GRacc) using threshold values. Acceleration data (in seconds) from the horizontal forward axis less than a threshold (-0.40 to -0.48 g for EX1, according to individuals; -0.50 g for EX2 for all cows) were computed as grazing time. For EX1, GRacc was compared to GRman. Results were calculated in terms of sensitivity (grazing time calculated from acceleration data correctly classified as grazing) and specificity (non-grazing time from acceleration correctly classified as non-grazing).

A large number of missing values for acceleration data in EX1 (72% to 99%; mean of 88%; 54 cow days), due to lost data packages, required pre-processing of data. Periods of less than 30 min. where data were missing were filled with, e.g. grazing, if the start and end of the period was computed as grazing; or with 50% of each activity if the start and end activities differed. After this correction, the percentage of missing values ranged from 0% to 95%, with an average of 20%. Only cow-days with less than 50% missing values were used for further analysis. In EX2, 50 out of 57 cow-days were available.

Statistical analyses were performed using the software R (R Development Core Team, 2011). Data were tested for outliers (outlierTest), homoscedasticity (bartlett.test) and normality (qqnorm and Shapiro.test). Analysis of variance was performed (lm or kruskal.test) and Spearman correlation test was used.

## **Results and discussion**

EX1.

Manual observations indicated an effect (P < 0.05) of the HA, according to stocking density: cows from the group Low (low HA, higher stocking density) spent less time grazing (37% ±9) than cows in group High (42%±7). A similar trend (P = 0.08) was observed for GRacc: cows from the group Low spent less time grazing (39% ±7) than cows in group High (42% ±6). Comparing all GRacc to all GRman values from EX1 indicated an average sensitivity of 74% (±15) and specificity of 82% (±8). These results are similar to other reported experiments with accelerometer measurements of grazing time (Moreau *et al.*, 2009). The correlations between GRacc and estimated intake were 0.16 (21 cow days), as compared to 0.59 (22 cow days) respectively for the Low and High group. Calculated grass intake on all pastures was low (av. 3.3 kg DM day<sup>-1</sup>) due to ad libitum feeding in the barn with TMR. EX2.

Results indicated a higher intake (P < 0.01) for cows pasturing on 'short' grass (H11): 15.0 (±1.4) vs. 13.8 (±2.4) kg DM for high grass (H16). The grazing time was also longer on the 'H11' grass than 'H16' grass (72% versus 65%). A reason could be that the shorter grass was more palatable (Gibb *et al.*, 1997), assuming most of the short grass is re-growth. The percentage of time spent grazing was higher than in Experiment 1, and more in accordance to what has been previously reported: Ueda *et al.* (2011) reported that

cows spent 67% of their time grazing when on pasture for 8 hours. The Spearman correlation test indicated a correlation between estimated intake and estimated percentage of grazing time of 0.52 (group H11; n = 17) and 0.82 (group H16; n = 7) for tightly attached sensors, and 0.28 (H11; n = 14) and 0.31 (H16; n = 12) for the loosely attached sensors (Figure 1). This indicates that estimation of grazing time is more accurate on H16 grass and a higher degree of correlation (and less variation) between intake and GRacc, when using tightly attached sensors.



Figure 1. Percentage of time spent grazing versus intake (kg DM) in EX2, according to sensor type (Tight: left panel, Loose: right panel) and grass height (H11, H16). Lines: fitted data from the corresponding linear models

#### Conclusions

Results of EX1 indicate that accelerometers can be used to estimate grazing time for cows on pasture. Some degree of correlation was found between grazing time and estimated grass intake on pasture. The highest correlations were found for cows pasturing in Experiment 1 for the high HA (rho = 0.59) and in Experiment 2 for the cows grazing in the rotational grazing system on a pasture with grass length of 16 cm (rho = 0.82).

#### References

Gibb M.J., Huckle C.A., Nuthall R. and Rook A.J. (1997) Effect of sward surface height on intake and grazing behavior by lactating Holstein-Friesian cows. *Grass and Forage Science* 52, 309–321.

Moreau M., Siebert S., Buerkert A. and Schlecht E. (2009) Use of a tri-axial accelerometer for automated recording and classification of goats' grazing behaviour. *Applied Animal Behaviour Science* 119, 158–170. Oudshoorn F.W. (2009) *Innovative technology and sustainable development of organic dairy farming*. PhD thesis Wageningen University, available on www.automaticmilking.dk

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Ueda Y. Akiyama F., Asakuma S. and Watanabe N. (2011) The use of physical activity monitor to estimate the eating time of cows in pasture. *Journal of Dairy Science* 94, 3498–3503.

## Voluntary dry matter intake in heifers from "Low Input" system from grasslands with different intensity of management

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### Abstract

This paper presents the results of tests of voluntary dry matter intake (VI) of silages made from grasslands with different intensity of utilization in 2010. Grasslands were managed in this way: medium intensive utilization (3 cuts per year,  $N_{90}P_{30}K_{60}$  per year); low intensive utilization (2 cuts per year,  $N_{90}P_{30}K_{60}$  per year); and extensive (control) utilization (2 cuts per year, nil-fertilization). The experiment was conducted on 2×6 heifers from the Low Input system using 2 types of housing: loose box housing (using feeding troughs – Roughage Intake Control (RIC)) and tie-stall housing (manual feeding). VI was influenced by intensity of grassland management (increasing intensity of use increased VI) and also by the housing system. Our results confirmed higher VI (P < 0.01) in medium intensive (19.3–20.9 g kg<sup>-1</sup> LW) and low intensive treatments (17.1–18.5 g kg<sup>-1</sup> LW) than in extensive treatment (15.1–17.9 g kg<sup>-1</sup> LW). Higher VI (P < 0.01) was confirmed in loose housing system (15.7–20.9 g kg<sup>-1</sup> LW) than in tie- stall housing system (15.1–19.3 g kg<sup>-1</sup> LW). There was a negative correlation between VI and the content of fibre (P < 0.01), as well as a positive correlation between the VI and the NEL (P < 0.01).

Keywords: voluntary intake, feeding value, housing system

## Introduction

Forages from permanent grassland are important components especially for the suckler cows' nutrition. However, the feeding value and VI of the forage are decisive factors for meeting the nutritional requirements of different animal categories and their feeding management. The aim of this study was to determine the VI of silages made from grasslands under different grassland management with two different housing systems: loose housing (LHS) and tiestall housing (TSH) in heifers from a suckler cow breeding system.

## Materials and methods

The feeding trials were conducted using silages made from the cuts of different grassland treatments. For the trials we used 2×6 heifers (6 heifers per group, 295–351 kg LW), crossbreeds of Czech Fleckvieh with 25% meat breeds (Limousin) from a suckler-cow rearing system. The first group used the loose housing system (4 feeding troughs with RIC management 1.7, Insentec B.V.). The second group used the tie-stall housing system (with manual feeding and weighing 2× per day). There were 5 feeding trials from January to April 2011. The experimental grasslands were managed as follows: fertilized medium intensive (3 cuts per year – first on 30 May followed by cuts at 60d intervals,  $N_{90}P_{30}K_{60}$  per year, only the first cut was tested for operational reasons); fertilized low intensive (2 cuts per year – first on 15 June, next cut after 90d,  $N_{90}P_{30}K_{60}$ per year) and extensive – control (2 cuts per year – first on 15 June with the next cut after 90d, nil-fertilization. Before each cut, the botanical composition and vegetation stage were evaluated. The grass silage was made by cutting grass and leaving it to dry naturally in the field, after which the dry matter content was about 38%. After wilting, the mowed fodder was compressed into round bales using a baling press with chopping equipment. The fermentation process lasted 3 months. Each trial was divided into a habituation period (10–14 days) and a testing period (10 days). The animals were offered grass silage *ad-libitum*. The spectrum of the nutrients (crude protein – CP, crude fibre – CF, ash, fat, neutral detergent fibre – NDF and acid detergent fibre – ADF) in silages was analysed. The *in vitro* organic matter digestibility (OMd) was determined by the method of Tilley and Terry (1963) modified according to Resch (1991). The energy and protein value was predicted by means of a regression equation (Pozdíšek *et al.*, 2003). Evaluation of the nutritive value in system NE (net energy), PDI (officially used in the Czech Republic) corresponded with INRA (Jarrige *et al.*, 1989). For the statistical evaluations of VI a linear regression model was used with mixed effect (using statistical program R, level of significance P < 0.05). For the statistical model the effect (mean value of parameters, intensity of utilization, CP, CF, NDF, housing system, LW, animal and residual error) was used.

#### **Results and discussion**

The botanical composition of grasslands is shown in Table 1. The dominant species were *Dactylis glomerata*, *Poa pratensis*, *Lolium perenne*, *Trifolium repens*, and *Taraxacum* sect. *Ruderalia*. In the first cut of the medium-intensive treatment there was an increase in the species *Trifolium pratense* (up to 26%) and a simultaneous decrease in the percentage of forbs (up to 16%). The weed species *Crepis biennis* slightly increased in dominance (up to 9%) which resulted in a slight decrease in the feed value and subsequently VI, as well. The low intensive and extensive treatment showed a low percentage of legumes (4–5%) and high percentage of forbs (31–53%). The species with low nutritional value (e.g. *Urtica dioica*, *Aegopodium podagraria*, *Anthriscus sylvestris*) were more dominant in those grasslands (2–8%). Buchgraber (2005) confirmed that these weed species decrease the VI and feed value of silages.

			•	<u> </u>					U						
TD	Cut	G	L	F	DM	CP	Fat	CF	NFE	Ash	ADF	NDF			
IK	Cut	% g kg <sup>-1</sup>						g kg <sup>-1</sup> DM							
MI	1.	57	27	16	321.7	117.6	37.3	287.5	471.3	86.3	334.5	553.8			
LI	1.	65	4	31	448.3	100.8	22.6	319.2	450.1	107.3	410.0	585.9			
LI	2.	53	5	42	354.1	117.8	28.8	300.4	406.6	146.4	325.9	541.9			
Е	1.	71	4	25	448.3	108.2	22.3	356.2	429.4	83.9	395.5	563.6			
E	2.	42	5	53	309.4	116.3	27.5	266.0	427.0	163.2	294.8	475.2			

Table 1. Botanical composition of grasslands and nutrient content of silages

TR = treatment: MI – medium intensive, LI – low intensive, E – extensive; G = grasses, L = legumes, F = forbs; NFE – nitrogen free extract, DM – dry matter.

Increases in the concentration of CF, NDF together with decreases in the concentration of CP, NEL and other nutrients were found in connection with reduced intensity of utilization. The same tendencies were reported by Buchgraber (2005), Pozdíšek *et al.* (2008) and Gruber *et al.* (2011). The VI of silages (Table 2) ranged from 20.9 g kg<sup>-1</sup> LW (LHS) and 19.3 g kg<sup>-1</sup> LW (TSH) for the medium intensive treatment, 18.4–18.5 g kg<sup>-1</sup> LW (LHS) and 17.1–17.4 g kg<sup>-1</sup> LW (TSH) for the low intensive treatment, and 15.7–17.9 g kg<sup>-1</sup> LW (LHS) and 15.1–17.3 g kg<sup>-1</sup> LW (TSH) for the extensive treatment. Krizsan and Randby (2007) found VI of grass silages (325–383 g kg<sup>-1</sup> DM of ADF concentration) at the level of 17.90–26.50 g kg<sup>-1</sup> BW in heifers (mean 150 kg BW). Our results are in line with Gruber *et al.* (2011), who studied the VI of grasslands under the same type of management in dairy cows. Pozdíšek and Kohoutek (1998) tested VI of four grass varieties conserved by freezing (190.6–368.5 g kg<sup>-1</sup> DM of CF) at the level of 24.8–30.6 g kg<sup>-1</sup> BW in heifers (mean 300 kg BW – tie stall housing). There was no significant difference (P > 0.05) between weight in using heifers in each group. The statistical model confirmed higher VI (P < 0.01) in medium intensive and low intensive treatment. Higher VI (P < 0.01) was confirmed

in the loose housing system than in the tie-stall housing system (all treatments) There were negative correlations between VI and the content of fibre and NDF (P < 0.01), as well as a positive correlation between VI and the energy concentration (P < 0.01).

			DMd LW LHS TSH					DDIN /		Voluntary dr			y matter intake		
TD	Cut	OMd			NEL	PDIN	PDIE	DDIE	Mean		SD		C	V	
IK C	Cui							FDIE	LHS	TSH	LHS	TSH	LHS	TSH	
		%	k	g	MJ.kg <sup>-1</sup> DM	g.kg <sup>-1</sup>	DM			g kg⁻	<sup>1</sup> LW		ç	%	
MI	1.	66.0	308	306	5.34	75.8	82.2	0.922	20.9	19.3	1.9	1.7	9.44	8.95	
LI	1.	61.5	300	295	4.80	64.8	75.3	0.860	18.5	17.1	0.7	1.5	3.97	8.73	
LI	2.	66.5	322	318	5.03	75.7	78.9	0.960	18.4	17.4	1.1	1.8	5.91	10.09	
Е	1.	56.4	351	348	4.45	68.9	73.8	0.934	15.7	15.1	1.4	1.2	8.67	7.84	
E	2.	70.9	315	312	5.34	75.1	80.9	0.928	17.9	17.3	1.5	1.7	8.14	10.03	

Table 2. Nutrient content and voluntary intake of silages by different grassland management

TR = treatment: MI – medium intensive, LI – low intensive, E – extensive; BW = mean body weight in group; SD = standard dev. per group; CV = coeff. of variation per group; LHS – loose housing; TSH – tie-stall housing, LW – live weight, OMd – organic matter digestibility.

#### Conclusions

Our results confirmed higher VI (P < 0.01) in medium intensive (19.3–20.9 g kg<sup>-1</sup> LW) and low intensive treatments (17.1–18.5 g kg<sup>-1</sup> LW) than in extensive treatment (15.1–17.9 g kg<sup>-1</sup> LW). Higher VI (P < 0.01) was confirmed in the loose housing system (15.7–20.9 g kg<sup>-1</sup> LW) than in the tie- stall housing system (15.1–19.3 g kg<sup>-1</sup> LW). The quality (chemical composition and nutrients contents) of fodder can be altered through grassland management, i.e. the number of cuts and fertilization. The VI of heifers is affected by the intensity of grassland management and the effect of housing system is also important in Low Input systems. Furthermore, study of the VI in different cattle categories and grassland management is needed.

#### Acknowledgement

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#### References

Buchgraber K. (2005) Kann die Futterqualität am Grünland gesteigert werden? In: Kohoutek A. *et al.* (eds) *Kvalita píce z travních porostů*, VÚRV, v.v.i., Prague, pp. 5–11.

Gruber L., Schauer A., Häusler J., Adelwöhrer A., Urdl M., Südekum K.H., Wielscher F. and Jäger R. (2011) Einfluss des Vegetationsstadiums von Wiesenfutter auf Verdaulichkeit, Futteraufnahme und Milcherzeugung. In: 38. Viehwirtschaftliche Fachtagung, 13–14 April 2011, Bericht LFZ Raumberg-Gumpenstein, pp. 43–65.

Jarrige R. (1989) Ruminant nutrition – recommended allowances & feed tables. INRA/John Libbey Eurotext-Editions Medicine-Sciences, Montrouge. 390 pp.

Krizsan J. and Randby A.T. (2007) The effect of fermentation duality on voluntary intake of grass silage by growing cattle fed silage as the sole feed. *Journal of Animal Science* 85, 984–996.

Pozdíšek J., Loučka R. and Machačová E. (2003) Digestibity and nutrition value of grass silages. *Czech Journal of Animal Science* 48, 359–364.

Pozdíšek J. and Kohoutek A. (1998) Voluntary intake of fresh forage of selected grass species conserved by freezing in cattle. *Czech Journal of Animal Science* 43, 349–354.

Pozdíšek J., Kohoutek A., Látal O., Svozilová M. and Štýbnarová M. (2008) Voluntary intake by cattle consuming forage from grasslands of different quality. In: Xie Haining, Huang Jiehua (eds) *Multifunctional Grasslands in a Changing World* – Volume II, Guangdong People's Publishing House, China, pp. 731–731. Resch R. (1991) In vitro Verdaulichkeitsuntersuchung nach Tilley und Terry, 1963. *Bericht über die Tagung der ALVA Fachgruppe Versuchswesen*. Innsbruck, pp. 41–42.

Tilley J.M.A. and Terry R.A. (1963) A two-stage technique for the invitro digestion of forage crops. *Journal of the British Grassland Society* 18, 104–111.

## Grazing effects on the quality and quantity of forage in Inner Mongolian grassland

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### Abstract

The efficiency of utilization of nutrients by ruminants for productive purposes is determined by various factors. In this study, the effect of years and grazing intensity on aboveground net primary production (ANPP) and on nutritional forage characteristics (i.e. the concentration and yield of crude protein (CP), cellulase digestible organic matter (CDOM), metabolizable energy (ME) and neutral detergent fibre (NDF)) were tested in the typical steppe of Inner Mongolia within a controlled grazing experiment during six consecutive years (2005–2010). Grazing effects were tested along a gradient of seven grazing intensities (ungrazed to very heavily grazed). Our results show that years and grazing intensity significantly affect the concentration and yield of forage nutritional parameters. Forage nutritional yields showed higher values in years with higher rainfall. Forage CP, CDOM and ME concentrations increased but ANPP and nutritional yields decreased with increasing grazing intensity. Furthermore, forage nutritional yields were positively correlated with the dominance of major species, but negatively or less correlated with species diversity.

Keywords: forage nutritional yields, forage quality, ANPP, grazing, species diversity, Inner Mongolia steppe

#### Introduction

The ability of grassland to provide forage for animal growth relies on its biomass production and its nutritive quality; both factors determine the forage nutritional yields (e.g. the crude protein yields (CPY g m<sup>-2</sup>)). Understanding forage nutritional yield dynamics in semi--arid grassland is crucial because the combined evaluation of grazing affecting ANPP and forage nutritional concentrations may offset the inverse effects of each other. The forage nutritional yields may provide important information about forage management systems and, consequently, contribute to grassland sustainability. In addition, grazing-induced shifts in species composition and species diversity may also exert profound effects on ecosystem function and thus forage quality. Therefore, the present study aims to analyse the effects of grazing intensity on aboveground net primary production (ANPP) and quality of forage (yield and concentration of nutritive parameters) during six consecutive years. The interactions of these factors with further botanical parameters (e.g. species diversity) were also analysed.

## Materials and methods

The grazing experiment was established in 2005 in Inner Mongolia Grassland Research Station. The average temperature and precipitation in the region is  $0.9^{\circ}$ C and 329 mm (1982–2010). A moderate grazed area of about 150 ha of natural grassland was fenced and the grazing intensity randomly distributed in paddocks (2 ha–sized each) in a randomized block design. Seven grazing intensities were tested: no grazing (0 sheep ha<sup>-1</sup>), very-light (1.5 sheep ha<sup>-1</sup>), light (3.0 sheep ha<sup>-1</sup>), light-moderate (4.5 sheep ha<sup>-1</sup>), moderate (6.0 sheep ha<sup>-1</sup>), heavy (7.5 sheep ha<sup>-1</sup>) and very heavy (9.0 sheep ha<sup>-1</sup>) grazing. Further details on grazing intensity classification are given in Schönbach *et al.* (2011). Within each

grazing plot, three grazing exclosures  $(3 \text{ m} \times 2 \text{ m})$  were set up randomly in June prior to the start of the grazing season. Biomass sampling was done monthly inside and outside the exclosures. After sampling, exclosures were removed to new, previously grazed locations to measure the biomass growth of each month and to calculate the annual aboveground net primary production (ANPP).

$$ANPP = ABg1 + (ABu2 - ABg1) + (ABu3 - ABg2) + (ABu4 - ABg3)$$
(1)

Indices g (grazed) and u (ungrazed) represent outside and inside the exclosure. The aboveground biomass (AB) was collected at sampling time i (from June to Sept., respectively). For quality analysis the collected plant materials were dried at 60°C, weighed, ground to pass a 1 mm sieve and scanned by using Near-Infrared-Spectroscopy (NIRS) for feed quality parameters determination. The annual weighted means of forage nutritional yields (FNY) were calculated by multiplying ANPP with forage nutrient concentration sampled from outside the exclosure cages and summing to obtain the feed quality on offer:

 $FNY = ANPP \times forage nutrient concentration$  (2)

Analysis of variance (ANOVA) processed with mixed model was applied by using 'grazing intensity', 'block', 'year' and their interactions as fixed effects and 'year' as a repeated effect, and Pearson correlation analysis was used to analyse the associations of forage nutritional yields and plant species composition and species diversity parameters (richness, diversity and evenness).

#### **Results and discussion**

The ANOVA analysis showed that 'year' significantly affected forage nutritional yields (Table 1). Grazing intensities had also significant effect on the forage nutritional yields by increasing the crude protein (CP) concentration from 99 to 149g kg<sup>-1</sup> DM (dry matter), cellulase digestible organic matter (CDOM) from 612 to 659g kg<sup>-1</sup>DM and metabolizable energy (ME) from 8.45 to 9.25 MJ kg<sup>-1</sup> DM, whereas ANPP decreased from 200 to 104 g DM m<sup>-2</sup> on average, along grazing intensities, from 2005 to 2010. The Pearson correlation analysis indicated that the forage nutritional yields were positively correlated with the biomass of Levmus chinensis (Trin.) Tzvel., Stipa grandis P. Smirn. and Carex korshinskyi (Kom.) Malyschev., but not correlated to species richness (except NDFY) and negatively correlated with species diversity (except NDFY) and evenness (Table 2). Our results demonstrated that the grazing intensity decreased the forage nutritional yields as a result of variations in forage nutritional concentration and ANPP. The increment in the forage nutritional concentration with increasing grazing intensities did not compensate for decreased ANPP, which finally led to reduced forage nutritional yields at the highest grazing intensities. We also observed that the forage nutritional yields were strongly determined by species diversity and composition (mainly by dominant species). Hooper and Vitousek (1997) pointed out that the ecosystem processes may be largely affected by the different identities in species composition if some of the species traits dominate. Since the research already confirmed that species diversity and species composition were affected by grazing intensities and years parameters, we can conclude that species diversity and composition are also good predictors of the dynamic of forage nutritional yields in Inner Mongolia natural grassland.
Avenue Steeling Date	0	15	2	15	6	75	0	(sheep
Average Stocking Kate	0	1.5	5	4.3	0	1.5	9	ha-1)
Grazing Intensity	0	1	2	3	4	5	6	S.E.M
		Forage	nutritive c	oncentrat	ions			
CP (g kg <sup>-1</sup> DM)	99d	96d	106cd	114c	107cd	131b	149a	4.7
NDF (g kg <sup>-1</sup> DM)	694ab	700a	699a	699a	702a	690ab	676b	2.7
CDOM (g kg <sup>-1</sup> OM)	612c	610c	614c	622bc	614c	639b	659a	5.6
ME (MJ kg <sup>-1</sup> DM)	8.45cd	8.41d	8.52cd	8.66c	8.52cd	8.90b	9.25a	0.1
		Forage r	nutritional	yields (F	NY)			
ANPP (g DM m <sup>-2</sup> )	200ab	212a	194abc	159cd	173bc	126de	104e	17.3
CPY (g m <sup>-2</sup> )	19.4a	19.7a	20.1a	18.2ab	18.1ab	16.5b	15.4b	1.1
NDFY (g m <sup>-2</sup> )	139.2ab	148.70a	136.2abc	111.2dc	121.6bc	86.7de	70.7e	12.6
CDOMY (g m <sup>-2</sup> )	121.8ab	128.4a	118.4ab	99.2dc	105.3bc	79.9de	68.4e	9.7
MEY (MJ m <sup>-2</sup> )	1.68ab	1.76a	1.64abc	1.38c	1.46bc	1.11d	0.96d	0.13
Grazing intensity: 0 11	arazed 1	very_li	aht: 2 lie	rht 3 li	aht_moder	ate / m	oderate.	5 heavy

Table 1. Effects of grazing intensity on the nutritive concentration and yields of forage

Grazing intensity: 0 – ungrazed; 1 – very-light; 2 – light; 3 – light-moderate; 4 – moderate; 5 – heavy; 6 – very-heavy.

a, b, c, d within rows, means followed by the same letters are not significantly different (P < 0.05).

Table 2. Spearman correlation coefficients (N = 84) between the forage nutritional yields and species richness (R), diversity index (D), evenness (E) and species composition

Yields	Ley	Sti	Car	Agr	Cle	Ach	Koe	R	D	Е
CP (g m <sup>-2</sup> )	0.31**	0.32**	0.22*	0.07ns	-0.24*	-0.09ns	0.08ns	0.12ns	-0.28**	-0.41***
NDF (g m <sup>-2</sup> )	0.59***	0.27*	$0.41^{***}$	0.14ns	-0.13ns	0.08ns	0.24ns	0.25*	-0.17ns	-0.34**
CDOM (g m <sup>-2</sup> )	0.55***	0.32**	0.36***	0.14ns	-0.18ns	0.05ns	0.23ns	0.19ns	-0.23*	-0.37***
ME (MJ m <sup>-2</sup> )	0.53***	0.33**	0.35**	0.14ns	-0.19ns	0.04ns	0.22ns	0.18ns	-0.24*	-0.38***
The abbreviation	ons are: I	Ley (Leg	ymus chi	nensis),	Sti (Stip	a grand	is), Ach	(Achna	therum s	ibiricum
(Linn.) Keng),	Agr (Agr	opyron	cristatum	(L.) G	aertn), C	le (Cleis	togenes	squarro	sa (Trin.	) Keng),
Car (Carex korshinskyi (Kom.) Malyschev.), Koe (Koeleria cristata (Linn.) Pers.).										

## Conclusions

Increasing grazing intensity leads to an increase in forage nutritional forage value and to a decrease in ANPP and, consequently, to a decrease of forage nutritional yields. The forage nutritional yields were positively correlated with the biomass production of the dominant species *L. chinensis*, *S. grandis* and *C. korshinskyi*, but not correlative to species richness and negatively correlated with species diversity and evenness. Species composition and species diversity may be used to predict the fluctuation of forage nutritional yields with grazing intensities along the time. They also affect grassland stability and the whole ecosystem function.

## References

Hooper D.U. and Vitousek P.M. (1997) The effects of plant composition and diversity on ecosystem processes. *Science* 277, 1302–1305.

Schönbach P., Wan H.W., Gierus M., Bai Y.F., Muller K., Lin L.J., Susenbeth A. and Taube F. (2011) Grassland responses to grazing: effects of grazing intensity and management system in an Inner Mongolian steppe ecosystem. *Plant and Soil* 340, 103–115.

# Milk performance of two cow breeds at two levels of supplementation in long residence time grazing paddocks

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# Abstract

Milk performance of four groups of cows (H0, H4, N0 and N4) was studied under grazing: two cow breeds, Holstein-Friesian (H) vs. Normande (N), supplemented at pasture with two levels of concentrate, low vs. high (0 and 4 kg cow<sup>-1</sup> day<sup>-1</sup>). A simplified rotational grazing system using three paddocks per rotation with a mean residence time per paddock of 10-days was examined. During this long residence time, maximum milk yield (MY max.) was reached at day 4 followed by a milk yield drop (Dm) at the end. Milk production was higher (P < 0.001) in Holstein-Friesian than in Normande cows (7,452 over 6,067 kg cow<sup>-1</sup> year<sup>-1</sup>). Supplemented cows produced more (P < 0.001) milk than unsupplemented cows (7,570 over 5,949 kg cow<sup>-1</sup> year<sup>-1</sup>). In each 10-day grazing cycle, supplemented cows had higher (P < 0.001) peak (MY max., 25.6 kg cow<sup>-1</sup>) and less (P < 0.001) drop (Dm – 5.3 kg cow<sup>-1</sup>) than those without concentrate (21.2 and – 5.7 kg cow<sup>-1</sup>). The peaks and drops of milk yield were higher (P < 0.001) in Holstein-Friesian (25.5 and – 6.2 kg cow<sup>-1</sup>) than in Normande cows (21.3 and – 4.7 kg cow<sup>-1</sup>). When long-residence grazing paddocks are used, these peaks and drops of milk yield are important factors to control for a good milk performance considering both the cow breeds and the supplementation effects.

Keywords: simplified rotational grazing system, residence time, milk peaks and drops, cow genotypes, supplementation effects

# Introduction

It is important to reduce management practices, number of paddocks, fencing and labour in farm conditions. Delaby *et al.* (2009) investigated during 5-years the whole lactation response of two cow breeds, Holstein-Friesian (H) vs. Normande (N), managed at two parities, primiparous vs. multiparous, applying the simplified rotational grazing system in Normandy (France) and using low rates of supplementation at grazing by feeding two levels of concentrate, 0 vs. 4 kg cow<sup>-1</sup> day<sup>-1</sup>. As part of this work, we focus our research in determining the maximum of milk yield (MY max.) and the drop of milk (Dm as the difference between the last-day milk and the max. milk yield in a paddock) in each grazing cycle for a long residence time in a paddock (on average 10-days), defined by Hoden *et al.* (1991), during the whole grazing season of 9-years in a similar experiment.

# Materials and methods

The study was conducted from 2001 to 2009 at the INRA experimental farm of 'Le Pin au Haras' in France. The location is a grassland zone with drained permanent pastures and sown pastures of perennial ryegrass either pure or associated to white clover.

*Experimental Design and Treatments.* In November of each year, a mean of 72 dairy cows (Table 1) were randomly assigned in a block design with a  $2\times2$  factorial arrangement of

four grazing treatments (H0, H4, N0 and N4). The experiment investigated the milk performance of two cow breeds, Holstein-Friesian (H) vs. Normande (N), under two grazing feeding regimes, without vs. with concentrate (0 vs. 4 kg cow<sup>-1</sup> day<sup>-1</sup>), managed at two parities, primiparous vs. multiparous, across successive lactations during a 9-year period. The objective was to determinate the MY max. in each grazing cycle and the Dm in both cow breeds for both grazing feeding regimes.

Table 1.	Number of	of selected	grazing	cycles b	y year	from	the	total	observed	during	a 9-year	period
and num	ber of cov	vs at differ	ent bree	ds, conce	entrate	levels	s and	l pari	ties			

					Holstein	-Friesian <sup>3</sup>	Norn	nande	Conce	ntrate <sup>4</sup>
Year	Total Cycles <sup>1</sup>	Selected Cycles <sup>2</sup>	Grazing Data	Number of Cows	Prim. <sup>5</sup>	Mult.	Prim.	Mult.	0 kg	4 kg
2001	27	5	340	68	19	16	14	19	34	34
2002	24	6	433	72	16	19	10	27	35	37
2003	26	5	338	68	15	21	13	19	33	35
2004	24	7	487	70	22	16	10	22	34	36
2005	27	5	368	74	14	20	16	24	38	36
2006	25	4	285	74	12	23	18	21	38	36
2007	30	5	369	76	19	17	13	27	40	36
2008	27	4	288	72	16	18	11	27	37	35
2009	28	4	288	72	12	18	21	21	36	36
Total	238	45	3196	646	145	168	126	207	325	321
Average	26	5	355	72	16	19	14	23	36	36

Total number of <sup>1</sup>observed and <sup>2</sup>selected grazing cycles by year; <sup>3</sup>Cow Breeds (Holstein-Friesian vs. Normande); <sup>4</sup>Concentrate Levels (0 vs. 4 kg cow<sup>-1</sup> day<sup>-1</sup>); <sup>5</sup>Parities (primiparous vs. multiparous).

*Grazing Management*. During each rotation, the same total area was allocated to the four grazing groups to obtain the same average stocking rate of 4.0 cows ha<sup>-1</sup> in spring and 2.2 cows ha<sup>-1</sup> in autumn. The decision to change paddock was made according to the evolution in milk yield profile of the group receiving 4 kg day<sup>-1</sup> of concentrate. The paddock was changed when milk production over the previous 3 days corresponded to 85–90% of the maximum milk yield value observed on the paddock (Hoden *et al.*, 1991). Cows without concentrate also changed paddock at this rate to get more severe grazing.

# **Results and discussion**

The highest milk performance was reached by H4 (8,407 kg cow<sup>-1</sup> year<sup>-1</sup>) and the lowest by N0 (5,401 kg cow<sup>-1</sup>) with the other two groups (H0, 6,497 and N4, 6,733 kg cow<sup>-1</sup> year<sup>-1</sup>) in between (Figure 1). Milk yield was higher (P < 0.001) in Holstein-Friesian cows than in Normande. Supplemented cows produced more (P < 0.001) milk than unsupplemented. No significant interactions were found between cow breeds, concentrate levels and parities on MY max. and Dm on the selected grazing cycles during a 9-year period (Table 2). The MY max. at day-4 and Dm at day-10 were higher (P < 0.001) in Holstein-Friesian (25.5 and – 6.2 kg cow<sup>-1</sup>) than in Normande cows (21.3 and – 4.7 kg cow<sup>-1</sup>). The group of cows fed concentrate at pasture (4 kg cow<sup>-1</sup> day<sup>-1</sup>) showed higher (P < 0.001) MY max. and slightly lower (P < 0.001) Dm (25.6 and – 5.3 kg cow<sup>-1</sup>) than unsupplemented cows (21.2 and – 5.7 kg cow<sup>-1</sup>). Multiparous cows (25.3 and – 6.1 kg cow<sup>-1</sup>) had higher MY max. (P < 0.001) at day-4 and Dm at day-10 (P < 0.001) than primiparous cows (21.5 and – 4.9 kg cow<sup>-1</sup>). Good control of Dm in long residence time grazing paddocks helped us to maintain milk reduction steady throughout the lactation.



Figure 1. An illustration of the within paddock milk profile and of the milk performance of two cow breeds at two levels of concentrate in 2006

Table 2. Effect of two cow breeds and two grazing feeding regimes on the maximum of milk yield and the drop of milk in each grazing cycle during a 9-year period

	-	-		-	-	-				
		Holstein	-Friesian <sup>3</sup>	Ν	Jormano	le		Signif	icance <sup>4</sup>	5
Item <sup>1</sup>	Parity <sup>2</sup>	$0 \text{ kg}^4$	4 kg	0 kg	4 kg	SEM	Gen.	Conc.	Par.	GxCxP
MY max.	Prim.	21.2ª	25.8 <sup>b</sup>	17.9°	21.1ª	0.15				
	Mult.	24.8ª	30.2 <sup>b</sup>	20.8°	25.2 <sup>d</sup>	0.12				
	Average	23.0ª	28.0 <sup>b</sup>	19.4°	23.2ª	0.14	***	***	***	ns
Dm	Prim.	$-5.8^{a}$	-5.4 <sup>b</sup>	-4.3°	-3.9 <sup>d</sup>	0.06				
	Mult.	$-7.0^{a}$	$-6.6^{b}$	-5.5°	-5.1 <sup>d</sup>	0.05				
	Average	$-6.4^{a}$	$-6.0^{b}$	-4.9°	-4.5 <sup>d</sup>	0.05	***	***	***	ns

<sup>1</sup>Item: MY max. (Maximum of milk yield) and Dm (Drop of milk); <sup>2</sup>Parity (primiparous vs. multiparous); <sup>3</sup>Cow Breeds (Holstein-Friesian vs. Normande); <sup>4</sup>Concentrate Levels (0 vs. 4 kg cow<sup>-1</sup> day<sup>-1</sup>); <sup>5</sup>Significance: SEM = Standard Error of the Mean; <sup>a-d</sup> Means within a row with different superscripts differ significantly (P < 0.05). ns = Not significant; \*\*\*P < 0.001; \*\*P < 0.01; \*P < 0.05.

## Conclusions

Milk yield drop was observed in all treatment groups at the end of the 10-days resident time when using a simplified rotational grazing system of three paddocks. Despite this cyclic variation, cows did not show any negative effect on milk performance over the whole lactation. The results point to the importance of controlling the drop of milk yield, while getting a good grazing in each long residence paddock, without penalizing milk performance of dairy cows.

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## References

Delaby L., Faverdin P., Disenhaus C., Michel G. and Peyraud J.L. (2009) Effect of feeding strategies on the Holstein and Normande dairy cows performance and their evolution during the lactation. *Animal* 3(6), 891–905. Hoden A., Peyraud J.L., Muller A., Delaby L. and Faverdin P. (1991) Simplified rotational grazing management of dairy cows: effects of rates of stocking and concentrate. *Journal of Agricultural Science* 116, 417–428.

# Effect of calving date and parity on milk performance of grazing dairy herds

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# Abstract

The rate of cow replacement is quite high on intensive dairy farms. This study considers the effect of the proportion of primiparous and the calving date, on milk performance of cows during three periods under grazing conditions. Individual groups of Friesian cows, combining spring *vs.* autumn calving and primiparous vs. multiparous cows, were evaluated for milk yield and quality during spring-summer grazing of grass-clover pastures. The primiparous group from spring-calving cows produced 30–35% less milk, with similar protein but higher fat content, than multiparous group. These spring-calving herds had 22–30% more milk than the autumn-calving herds. The primiparous autumn-calving cows produced 10–15% less milk than the multiparous autumn cows or the primiparous spring cows. These differences were reduced when the milk yield of cows decreased as the season advanced or as concentrate was reduced. Good control of herd composition, concerning parity and the percentage of cows' replacement, would help to match feeding requirements with getting the best milk performance of grazing cows.

Keywords: stage of lactation, primiparous, multiparous, dairy cattle

# Introduction

Intensive dairy systems are predominant in the humid grassland region of Galicia (NW Spain), using high milk producing cows (9–10 t cow<sup>-1</sup> year<sup>-1</sup>) with high levels of concentrate (3–4 t cow<sup>-1</sup> year<sup>-1</sup>) to the detriment of more grazing-based systems. The low milk price, the end of European Union milk quotas in 2015 and the increasing input costs are focusing farmers on sustainable grazing dairy systems. Such systems are under study in the CIAM, with lower productivity cows (6–7 t cow<sup>-1</sup> year<sup>-1</sup>) and moderate rates of concentrate (1–2 t cow<sup>-1</sup> year<sup>-1</sup>) and longer life per cow (5–6 lactations). That means systems with a 20% rate of annual replacement, whereas intensive dairy systems reach a 40-50% of replacement, with only 2-3lactations per cow (González-Rodríguez et al., 2010). The available research on management methods for relocated dairy animals to allow them to optimize their productivity is very limited (Hefner, 2000). The composition of the herd should now be taken into account as being a very important factor influencing nutritional needs and milk performance of cows. The lactation stage of animals, days in milk, and the number of primiparous cows in the herd, with lower milk yield but similar feeding requirements than multiparous cows, was considered in our sustainable grazing dairy systems. The aim of this study was to evaluate the effect of the number of primiparous vs. multiparous cows and the number of days in milk, spring vs. autumn calving cows, on the milk performance of dairy cows at pasture.

# Materials and methods

A total of 93 Holstein-Friesian cows were evaluated during the spring and summer grazing in 2009 at CIAM experimental farm in NW Spain, using a low rate of concentrate, in five individual herds, according to its calving date and parity. Sixty autumn (A) calving cows (end October) had 5 kg grass dry matter silage supplement per cow, and 5 kg of concentrate at autumn grazing, and were housed in the winter period with 10 kg grass silage ration, from December until mid March. At

that time, the autumn cows turnout to grazing grass-clover swards as three independent groups' treatments: two of multiparous cows, with concentrate A-4 (n = 20), 4 kg per cow, or without it A-0 (n = 20), and one group of primiparous, PA (n = 20), also without concentrate. The other two groups under study were spring (S) calving cattle (mid March): either multiparous, S (n = 18), or primiparous groups, PS (n = 15), both calving and grazing in spring with 6 kg cow<sup>-1</sup> year<sup>-1</sup> of concentrate. After mid May the PS group had no concentrate. The grazing season was divided into three periods according to pasture quality: two periods in spring, (1) vegetative from March to mid May, (2) reproductive from May to end of July and one period in summer (3) from August to end of September. Graph with the standard error of the mean are performed by ANOVA with cows as random effects.

#### **Results and discussion**

The spring-calving group of multiparous (S) cows reached a milk peak of 38.8 kg cow<sup>-1</sup> in mid April, while the primiparous spring (PS) group reached 26.7 kg cow<sup>-1</sup> at the end of April (Figure 1). The best quality grass with 159 g kg<sup>-1</sup> of crude protein was recorded in the first spring grazing vegetative period (1) yielding a mean of 36.7 kg cow<sup>-1</sup> of milk for multiparous (S) and 25.7 kg cow<sup>-1</sup> for primiparous (PS) herds, both with similar milk protein content, 31.0 g kg<sup>-1</sup>, but different fat content, 35.5 and 37.2 g kg<sup>-1</sup>, respectively. In the second spring-grazing period (2), a more stemmy pasture had 142 g kg<sup>-1</sup> of protein; milk yield was 28.7 and 19.3 kg cow<sup>-1</sup> (33% more) in the multiparous (S) than primiparous (PS) cows, with similar milk protein, 31.2 g kg<sup>-1</sup>, and fat content, 38.1 g kg<sup>-1</sup>g in both groups. In the third grazing period (3), the protein content of pasture dropped to 106 g kg<sup>-1</sup> and grass growth was restricted by summer drought. Then, grazing was supplemented with grass silage, 5 kg cow<sup>-1</sup> day<sup>-1</sup>. The multiparous cows (S) yielded 23.4 kg cow<sup>-1</sup> with 4 kg cow<sup>-1</sup> day<sup>-1</sup> of concentrate, 35% more milk than the primiparous group (PS) with 15.2 kg cow<sup>-1</sup> with the same silage ration but without concentrate. Both groups, S and PS, had similar protein and fat content, 33.0 and 39.1 g kg<sup>-1</sup>, respectively.

The three autumn-calving groups, with 110 more days in milk and grazing during the spring vegetative period (1), are producing 28.2 kg cow<sup>-1</sup> for the (A-4) herd with concentrate, 23% less milk than the similar spring-calving multiparous group. The autumn groups, multiparous (A-0) and primiparous (PA), both without concentrate, were yielding 24.8 and 21.9 kg cow<sup>-1</sup>, 32% and 22% less milk than the spring-calving cows. Milk protein was similar (30.0



Figure 1. Milk yield in five groups of dairy cows: two spring calving cows, multiparous (S) and primiparous (PS), with concentrate and three autumn calving groups, two of multiparous, with concentrate (A-4) and without it (A-0), and one primiparous (PA) without concentrate, grazing in three periods at humid NW Spain



Figure 2. Milk quality, fat (white) and crude protein (dark bars) content in five groups of spring calving dairy cows, S and PS, and autunm calving cows, A-4, A-0 and PA, grazing in three periods: 1) spring vegetative, (2) spring reproductive and (3) summer at humid NW Spain

g kg<sup>-1</sup>) in the three autumn groups but with different fat content (37.6, 41.7 and 50.7 g kg<sup>-1</sup>, respectively). The higher fat content under similar grazing conditions was observed in the groups with lower milk yield. The milk yield was 12% higher in the A-0 than the PA group. The differences in milk of around 10% during the first period were maintained during the second, with 17.1 and 15.8 kg cow<sup>-1</sup>, and in the third grazing period, with 15.8 and 14.0 kg cow<sup>-1</sup>, for A-0 and PA respectively. When lower milk yield was produced, the differences between primiparous and multiparous tend to decrease. The autumn calving groups with no concentrate in the 2 and 3 periods had similar milk quality, 29.0 g kg<sup>-1</sup> of protein and 37.6 g kg<sup>-1</sup> of milk fat content (Figure 2).

The biggest differences in milk yield, 30–35%, were found between spring-calving primiparous (PS) and multiparous (S) cows at high producing early lactation. Also, these spring-calving cows had 22–30% more milk than the autumn-calving cows, under the same spring-grazing conditions. These differences were reduced when herds produced less milk towards the end of lactation or when they received less concentrate. All the different grazing treatments maintained a good sward quality as the main forage feed. Farmers are aware of the different milk yields from the spring – or autumn-calving herds, but rarely about the lower yield of primiparous cows, when the level of replacement is quite high as normally happens on the intensive dairy systems. Using these data for comparing herds, if we consider 20% versus 40% of primiparous, a difference of 400 kg of milk per cow was found, assuming a spring-calving lactation of 7500 kg cow<sup>-1</sup> year<sup>-1</sup>. The difference in milk decreased to 260 kg cow<sup>-1</sup> in the herd with the lower number of primiparous cows, considering the autumn-calving cows of 6300 kg per lactation. If these autumn-calving herds had no concentrate during the spring grazing, this difference was reduced to 82 kg cow<sup>-1</sup> for a 5600 kg per-lactation herd.

## Conclusions

Increasing the percentage of primiparous cows in the herd brings a reduction in milk yield, up to levels of 35% in the first third of lactation during spring grazing. These differences are reduced when the individual yields of cows decreased, comparing spring – versus autumncalving cows or reducing concentrate levels. The herd composition, parity and number of cow replacements, should be considered for improvement of feeding conditions and milk performance at grazing.

## References

González-Rodríguez A., Roca-Fernández A.I. and Vázquez-Yáñez O.P. (2010) Alimentación de las vacas lecheras en las zonas húmedas españolas. *Ganadería* 67, 54–60.

Hefner D.L. (2000) *Feeding management for cows entering a new herd*. In: Eastridge M.L. and Wayne F.T. (eds), Proceedings Tri-state dairy nutrition conference. USA, 183–192.

# Managing sward height throughout the year to increase forage accumulation

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# Abstract

Two management strategies for *Brachiaria decumbens* pasture under continuous stocking were evaluated in Vicosa, Minas Gerais, Brazil. In one management strategy, the grass height was kept at 25 cm throughout the entire trial. In the other, the pasture was kept at an average height of 15 cm during the winter, then changing to 25 cm from the beginning of spring until the summer. All pastures were grazed by cattle with variable stocking rates. The experiment was carried out using a randomized block design with four replications and subdivided plots. Swards kept at 25 cm throughout the trial had a lower average growth rate than swards maintained at 15 cm in the winter. Forage accumulation, from winter to summer, was 15% greater in pastures maintained at 15 cm in winter in relation to those with 25 cm. Winter was the season with the lowest rates of growth and herbage accumulation. Spring was the season with the highest rate of leaf senescence. To increase forage accumulation, *B. decumbens* must be kept under continuous stocking at 15 cm height in winter and 25 cm in spring and summer.

Keywords: Brachiaria decumbens, grazing, growth, leaf senescence, tiller

# Introduction

In Brazil, forage grasses of the genus *Brachiaria* are most commonly used in the formation of pastures. The species *Brachiaria decumbens* is especially represented in the areas cultivated with pasture used for ruminant production (Macedo, 2004). The key characteristics that justify the use of this forage are: suitable adaptation to tropical soil and climate, high competitive capacity, and large flexibility in its use and management. Most of the pasture systems in Brazil with *B. decumbens* are managed under continuous cattle grazing. Therefore, understanding the growth and senescence of plants throughout the seasons of the year and in pastures under different defoliation regimes makes it possible to identify appropriate grazing management strategies (Santos *et al.*, 2010). This work was carried out to determine whether the reduction of sward height throughout the winter season results in increased herbage accumulation in *B. decumbens* pastures.

# Material and methods

From June 2008 to March 2009, two management strategies of a *B. decumbens* pasture were evaluated at the Animal Science Department of the Federal University of Vicosa, located in the State of Minas Gerais, Brazil. The experimental site was located at an elevation of 651 m, 20°45' S and 42°51' W. Annual precipitation is approximately 1,340 mm and the average relative humidity is 80%. Maximum and minimum temperatures are 22.1°C and 15.0°C, respectively. The experimental area consisted of eight plots (experimental units) of 0.3 ha each. Pastures were managed under continuous stocking and with variable cattle stocking rates. In one management strategy, *B. decumbens* pasture was maintained at 25 cm height during the entire trial. In the other, the pasture was kept at an average height of 15 cm during the winter (July to September 2008), and at 25 cm from the beginning of winter until the

summer (October 2008 to March 2009). For maintenance of swards heights, cattle of about 200 kg were used. The experiment was carried out using a randomized block design with four replications and subdivided plots. Both management strategies for the pasture correspond to the plots. The seasons of the year are the subplots. The average stocking rates were 2.5 animal units ha<sup>-1</sup> in spring and 3.2 animal units ha<sup>-1</sup> in summer. In winter, the cattle were removed from the paddocks to maintain the desired sward heights. Estimates of leaf and stem growth, leaf senescence, and herbage accumulation were made using 16 marked tillers per experimental unit, according to the technique described by Bircham and Hodgson (1983). In order to express the rates of growth and senescence of leaf blades and stems in kg of dry matter ha<sup>-1</sup> day<sup>-1</sup>, conversion factors were generated. Data were analysed by analysis of variance with repeated measures over time and two treatments (management strategies) and three time periods (seasons). Data regarding the management strategies of the pasture were compared using the F-test, while the ones regarding the seasons of the year were compared by Tukey's test (10%).

#### **Results and discussion**

The growth rate of leaf plus stem was lower (P < 0.10) in the winter, intermediate in the spring, and higher in the summer (Table 1). Only 2.4% of the total growth of *Brachiaria decumbens* occurred during the winter, whilst in spring and summer these values were 42.8% and 54.8%, respectively.

Sward height (cm)		Seasons of the year	•	Maaa
Sward neight (cm)	Winter	Spring	Summer	Mean
	Growth ra	te (kg ha <sup>-1</sup> day <sup>-1</sup> of	dry matter)	
25	5.8	100.0	140.5	82.1B
15-25	7.0	125.8	152.1	95.0A
Mean	6.4c	112.9b	146.3a	
25	16.72	26.44	15.70	19.62A
15-25	9.36	18.39	12.78	13.51B
Mean	13.04b	22.42a	14.24b	
	Herbage accumul	ation rate (kg ha-1 d	ay <sup>-1</sup> of dry matter)	
25	-10.9	73.6	124.8	62.5B
15-25	-2.3	107.4	139.3	81.5A
Mean	-6.6c	90.5b	132.0a	

Table 1. Leaf and stem growths rates, leaf senescence rate, and herbage accumulation rate (kg ha<sup>-1</sup> day<sup>-1</sup> of dry matter) of *Brachiaria decumbens* pastures managed under continuous stocking and fixed (25 cm) or variable (15–25 cm) height during the seasons of the year

For each characteristic, means followed by the same letter in lowercase in the same line or in uppercase in the same column do not differ (P > 0.10).

The reduced availability of growth factors in the winter explains the lower growth rate during that season. In addition, lowering sward height to 15 cm in the winter resulted in a higher (P < 0.10) growth rate when compared with pastures maintained at a fixed height (25 cm) for the entire period. This result can be attributed to the intense renewal of tillers, especially in the spring, which occurred on *B. decumbens* pasture with 15 cm during the winter (Santos *et al.*, 2010). The leaf senescence rate was higher in the spring (P < 0.10), even with the pasture kept at 25 cm. A larger leaf senescence rate in the spring can be explained by tiller renewal during the spring (Santos *et al.*, 2010). It is possible that the leaves started senescence immediately in order to supply nutrients via translocation,

and thus help the expansion of new leaves during the spring. The grass kept lower in the winter probably had a higher energy balance, which explains its lower leaf senescence rate. The herbage accumulation rate increased (P < 0.10) from winter to summer. Moreover, the management strategy to maintain *B. decumbens* pasture with 15 cm in the winter also resulted in a greater (P < 0.10) herbage accumulation rate. From winter to summer, the reduction of *B. decumbens* pasture heights in early winter to 15 cm resulted (P < 0.10) in higher herbage accumulation (25.6 Mg ha<sup>-1</sup> of dry matter) than those maintained at a fixed height at 25 cm throughout all seasons (22.2 Mg ha<sup>-1</sup> of dry matter).

# Conclusions

In *B. decumbens* pastures, winter is the season with the lowest rates of growth and herbage accumulation, whereas spring is the season with the highest rate of leaf senescence. The *B. decumbens* pasture with 15 cm in winter and 25 cm in spring and summer has a higher forage accumulation, compared with 25 cm in all seasons.

# References

Bircham J.S. and Hodgson J. (1983) The influence of sward condition on rates of herbage growth and senescence in mixed swards under continuous stocking management. *Grass and Forage Science* 38, 323–331. Macedo N.C.M. (2004) Análise comparativa de recomendações de adubação em pastagens. In: Pedreira C.G.S., Moura J.C. and Faria V.P (eds.) Simpósio sobre Manejo da Pastagem, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, SP. pp. 317–356.

Santos M.E.R., Fonseca D.M., Nascimento Júnior D., Gomide C.A.M. and Gomes V.M. (2010) Tillering dynamics in *Brachiaria decumbens* pastures under continuous stocking. *Grassland Science in Europe* 23, 934–936.

# Diet selection under full grazing of two Holstein strains in organic farming

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# Abstract

The n-alkane profiles of plants and faeces were used to estimate the diet composition on pasture of two Holstein cow strains (New Zealand ( $H_{NZ}$ ) and Swiss Holstein ( $H_{CH}$ )). Additionally, grazing time around dung patches was determined by observation. No differences existed concerning herbage intake (12.2 vs. 12.3 kg 100 kg<sup>-1</sup> metabolic body weight (BW<sup>0.75</sup>), P = 0.89) and feed efficiency (1.35 vs. 1.42 kg energy corrected milk kg<sup>-1</sup> intake, P = 0.53). The  $H_{NZ}$  tended to ruminate for longer (446 vs. 412 min, P = 0.08) than  $H_{CH}$  cows, whilst grazing times were similar (573 vs. 615 min, P = 0.14) for both strains. Regarding diet composition (74 vs. 72% *Lolium perenne*, P = 0.74; 26 vs. 24% *Trifolium repens*, P = 0.74; 0% *Dactylis glomerata* and *Taraxacum officinale*) and the grazing time around dung patches (9 vs. 7 sec min<sup>-1</sup>, P = 0.20) no differences between the two cow types were detected. The method used to estimate diet composition of dairy cows during grazing on mixed-species pasture is promising. To obtain more reliable results, other non-digestible plant components, in addition to n-alkanes, should be considered for better differentiation of plant species.

Keywords: diet selection, pasture, Holstein strains, organic farming

# Introduction

In organic milk production, grass-clover pasture confers benefits such as  $N_2$  fixation, reduction of weed invasion and higher yields compared to a grass monoculture (Lüscher *et al.*, 2010). To improve the efficient use of mixed swards, understanding diet selection and managing preference during grazing is needed. Several studies indicate that differences occur in relation to herbage intake and grazing behaviour between cow types (McCarthy *et al.*, 2007; Schori and Münger, 2010; Kunz *et al.*, 2010).

The present study attempted to describe dietary selection through n-alkane profiles in plants and faeces of dairy cows grazing mixed-species pasture.

# Materials and methods

Twelve pairs of New Zealand ( $H_{NZ}$ ) and Swiss Holstein ( $H_{CH}$ ) from the herd of the organic farm 'La Ferme de l'Abbaye' (46°39'N, 7°3'E, 824 m a.s.l.) were selected for this study. The average lactation number was 2.3 and 2.5 for  $H_{CH}$  and  $H_{NZ}$ , respectively, and the cows were more than 150 d in milk, on average. Full-time rotational grazing without supplementation, except salt, was practised. Milk yield and BW were automatically recorded twice daily. Herbage intake on pasture was estimated with 6 pairs during week-33 (W1) and on the remaining 6 pairs during week-34 (W2). The double-marker method with the n-alkanes dotriacontane and tritriacontane was used (Mayes *et al.*, 1986). Beginning 5 d before the start of faeces collection, 910 mg d<sup>-1</sup> of dotriacontane were administered to each cow via gelatine capsules twice daily. During 5 d, spot samples of faeces were collected indoors daily. Herbage sampling on pasture in the morning started and stopped 1 d earlier than faeces collection. During each collection period three cow pairs were equipped with recorders (IGER Behaviour Recorder (Rutter *et al.*, 1997)) over 4 d to survey eating behaviour. To investigate the diet selection the profiles of n-alkanes in plants and faeces were evaluated with a non-negative least squares algorithm (Dove and Moore, 1995). For this purpose, n-alkanes with carbon chain lengths of 27 to 31, 33 and 35 to 36 atoms in frequently occurring plants like *Dactylis glomerata*, *Lolium perenne*, *Taraxacum officinale* and *Trifolium repens* were analysed. In addition, the time cows grazed around dung patches on pasture were measured.

For statistical analysis (Systat 12, Systat Inc., Chicago, USA), a two-way, univariate analysis of variance was applied. The parameter 'time grazing around dung patches' was evaluated using a mixed model.

## **Results and discussion**

The average herbage quality during the first and second collection periods, respectively, can be rated as good, as the following values per kg DM confirm: 191 and 179 g crude protein, 326 and 395 g neutral detergent fibre, 240 g and 272 g acid detergent fibre and 6.4 and 6.2 MJ net energy lactation.

Alkane profiles are shown for the four most common forage plants in the grazed pasture (Figure 1). *L. perenne* had the highest alkane concentration and *T. officinale* the lowest.



Figure 1. Alkane profiles of analysed plants (L. perenne scaled to 250 mg kg DM<sup>-1</sup>)

Table 1. Body	weight,	milk	yield,	intake,	time	spent	eating,	time	spent	ruminating	and	diet	selec-
tion of Holstei	in dairy c	cows											

	Н	СН	Н	NZ	SE		Р	
	W 1	W 2	W 1	W 2		С	W	C×W
Body weight, milk yield and in	ntake (k	<b>g</b> )						
Body weight	575	645	514	582	18	**	**	-
Milk yield	21.9	22.5	16.1	19.6	1.6	*	_	_
Energy corrected milk (ECM)	20.4	20.6	17.3	19.0	1.3	Т	_	—
Grass intake (DM d <sup>-1</sup> )	17.0	13.0	14.8	12.6	1.0	_	**	-
Intake BW-0.75 (kg 100 kg-1)	14.5	10.1	13.8	10.7	0.8	_	***	-
ECM intake <sup>-1</sup> (kg kg <sup>-1</sup> )	1.2	1.6	1.2	1.5	0.1	_	**	_
Grazing behaviour (min d <sup>-1</sup> )								
Grazing	636	594	575	570	26	_	_	-
Rumination	374	450	389	503	17	Т	***	-
Diet selection (%)								
Lolium perenne	62	82	68	81	6	_	*	-
Trifolium repens	38	18	32	19	6	_	*	_

Standard error of estimate (SE), cow type (C), week (W), metabolic body weight (BW<sup>0.75</sup>); – not significant, t tendency P < 0.1, \* significant P < 0.05, \*\* significant P < 0.01 und \*\*\* significant P < 0.001.

Table 1 shows the weight, milk production, intake, time spent eating and ruminating and the diet selection of the cows.  $H_{NZ}$  were lighter (P = 0.002), produced less milk per cow (P = 0.01) and the output of energy-corrected milk tended to be lower (P = 0.09) than with

 $H_{CH}$ . No differences existed concerning herbage intake per BW<sup>0.75</sup> (P = 0.89) and feed efficiency (P = 0.53). All these findings are consistent with Schori and Münger (2010). The grass intake differed only numerically between the two Holstein strains (P = 0.23), in contradiction to the more extensive study of Schori and Münger (2010). The cows were grazing approximately 10 h d<sup>-1</sup> with no difference (P = 0.14) between the cow types, in contradiction to McCarthy *et al.* (2007), but confirming Schori and Münger (2010). The H<sub>NZ</sub> tended to ruminate longer (P = 0.08) than H<sub>CH</sub>, but with no effect on feed efficiency.

The evaluation of the alkane profiles of plants and cow faeces leads to the conclusion that only *L. perenne* and *T. repens* were eaten on the pastures. Hence, *D. glomerata* and *T. of-ficinale* were avoided. These results have to be critically interpreted, because *T. officinale* was present in the pastures and is assumed to be at least partially eaten. The same applies – to a lesser extent – to *D. glomerata*. The low alkane concentrations and similar profiles might be the reason why *T. officinale* and *D. glomerata* do not appear in the results. The method used to estimate diet composition of dairy cows grazing mixed-species pasture is promising. To obtain more reliable results, in addition to alkanes, other indigestible plant components like long-chain alcohols and fatty acids should be considered for better differentiation of plant species. Accounting for the alkane profile in the remaining species fraction could as well improve the validity of this method.

In contrast to Kunz *et al.* (2010), no differences were detected between the two Holstein strains regarding the time spent grazing around dung patches (9 vs. 7 sec min<sup>-1</sup>, standard error of estimate 5.9, P = 0.20). The role of factors like genetics, acquired behaviour, herbage allowance, etc., which affect the eating behaviour of the dairy cows have to be further explored, in order to improve efficient utilisation of mixed-species pastures.

## Conclusions

The method used to estimate diet composition of dairy cows grazing on mixed-species pasture is promising. To obtain more reliable results, other indigestible plant components, in addition to the alkanes, should be considered for better differentiation of plant species. Other factors which may affect the grazing behaviour of dairy cows, like genetics, acquired behaviour, herbage allowance etc. have to be further explored in order to increase the efficient utilisation of mixed-species pastures.

# References

Dove H. and Moore A.D. (1995) Using a least-squares optimization procedure to estimate botanical composition based on the alkanes of plant cuticular wax. *Australian Journal of Agricultural Research* 46, 1535–1544. Kunz P., Wetter A., Roth N. and Thomet P. (2010) Comparison of the eating behaviour of two cow types on pasture. *Book of Abstracts 61st Annual Meeting of the European Association for Animal Production* 61, 30. Lüscher A., Huguenin-Elie O. and Suter D. (2010) Grass-clover mixtures: sward composition and diversity effects. *NJF Report* 6, 53–58.

Mayes R.W., Lamb C.S. and Colgrove P.M. (1986) The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. *Journal of Agricultural Science* 1007, 161–170.

McCarthy S., Horan B., Rath M., Linnane M., O'Conner P. and Dillon P. (2007) The influence of strain of Holstein-Friesian dairy cow and pasture-based feeding system on grazing behaviour, intake and milk production. *Grass and Forage Science* 62, 13–26.

Rutter S.M., Champion R.A. and Penning P.D. (1997) An automatic system to record foraging behaviour in free-ranging ruminants. *Applied Animal Behaviour Science* 54, 185–195.

Schori F. and Münger A. (2010) Grazing behaviour and intake of two Holstein cow types in a pasture-based production system. *Grassland Science in Europe* 15, 895–897.

# Combining grazing and automatic milking

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# Abstract

Grazing in combination with automatic milking is experienced as difficult and is not generally practised. This paper provides data on the combination of grazing and automatic milking in Europe. Furthermore, it presents opportunities and challenges for the combination of grazing and automatic milking, based on results from on-farm participatory research in the Netherlands. The main issues of economy, labour, grazing system, frequency of visits and infrastructure of the farm have been studied. In general, problems with grazing and automatic milking can be solved leading to an economically attractive activity.

Keywords: automatic milking, automatic milking systems (AMS), grazing

# Introduction

Over the next ten years we expect a sharp increase in the number of farms with automatic milking systems (AMS; milking robots) in Europe. Furthermore, we expect that grazing of dairy cattle is more and more appreciated by society, and therefore desired in the farm management. Grazing in combination with automatic milking is possible; this has been proven in many projects (e.g. Wiktorsson and Spörndly, 2002). However, it is experienced as difficult (e.g. Parsons and Mottram, 2000) and is not generally practised in Europe. In many countries grazing stops as soon as AMS are introduced (Van den Pol-van Dasselaar *et al.*, 2008). This study aims to provide insight on actual data on AMS and grazing in Europe. Furthermore, it presents opportunities and challenges for the combination of grazing and automatic milking, based on results from onfarm participatory research in the Dutch project 'Koe&Wij' (Cows and Us) (www.koeenwij.nl).

# Materials and methods

Statistical data on the combination of grazing and AMS in Europe are not easily available. Most countries have no statistical data on grazing on farms with AMS. Often also the general percentage of grazing animals is not recorded. Therefore, to obtain insight on the state-of-the-art with respect to grazing and AMS, a survey among EGF members of the Working Group 'Grazing' was carried out in October and November 2011. The members were asked to provide either statistical data or an educated guess on the general amount of grazing in their country and the amount of grazing on farms with an AMS.

Furthermore, main difficulties with respect to grazing and AMS were studied using on-farm participatory research in the Dutch project 'Koe&Wij'. Koe&Wij worked during three years with four groups of 15 dairy farmers located throughout the Netherlands in situations where grazing was experienced as difficult. This paper reports on the results of the 'group AMS'. In the 'group AMS' the main issues of economy, labour, grazing system, frequency of voluntary visits to the AMS and infrastructure of the farm were studied. Data were recorded on farm and analysed in the group.

# **Results and discussion**

There are hardly any milking robots in the south and east of Europe. Table 1 shows the percentage of grazing in different countries in north-western Europe (expressed as % of

all animals). Furthermore, it shows the percentage of grazing for animals milked with AMS only. Of course there is large variation between countries. Even though the data are often only an educated guess, it is clear that, on average, grazing is practised less on farms with an AMS. Results from Ireland, Sweden and Denmark are notable. In Ireland, grass-based seasonal systems dominate. This is also true on the few farms with an AMS. In Sweden, due to welfare legislation animals have to be given access to pasture, which explains the 100% grazing. In Denmark, there is a large difference between organic farms and conventional farms. Less than 5% of the conventional AMS herds graze. But also about one-fifth of the organic farms have an AMS and they will all practise grazing.

Country	All animals	AMS only
Ireland	99	99
the Netherlands	70–75	40
Belgium	85–95	10
Northern Germany	30	2
France	92	1
Sweden	100	100
Denmark	30–35	25
Switzerland	85-100	50
Austria	25	5

Table 1. Grazing animals, expressed as % of all animals (both AMS and conventional milking) and as % of animals being milked with AMS, in different EU-countries

The main difficulties of combining automatic milking with grazing were defined by the 'group AMS' of Koe&Wij as: economy, labour, grazing system, frequency of voluntary visits to the AMS and infrastructure of the farm. Since grazing is experienced as more difficult, many farmers believe that it is not economically profitable. However, data from accountancy agencies, which were shared in the project Koe&Wij, showed that the total profit of farms with an AMS and grazing was 3.5 to 7 Euro per 100 kg milk produced more than the total profit of farms with AMS and no grazing.

Farmers fear more labour when combining AMS and grazing. Table 2, which is based on estimates of the farmers in the 'group AMS', shows that in a situation of AMS and grazing considerably more time will be spent on milking (29 minutes day<sup>-1</sup> during the grazing season, fetching cows inclusive). Less time is needed for feeding (17 min day<sup>-1</sup> less) and taking care of the cows (cow management) (17 min day<sup>-1</sup> less). In total, about 5 min day<sup>-1</sup> less time is needed for daily management during the grazing period. Next to this, farmers estimate that the labour required for non-daily management activities like ensiling grass is, in total, also less in a grazing situation. Based on a grazing period of approximately 180 days, in the Netherlands a farmer with an AMS and grazing needs 30-35 h<sup>-1</sup> yr<sup>-1</sup> less labour. The grazing system of farms with an AMS differs from the farms with a conventional milking system. The 'group AMS' experienced a system of unrestricted grazing as difficult. A system of restricted grazing led to a high grass intake and a high milk production. Continuous grazing was seen as the optimal grazing system. In this system, the herbage allowance, grass intake and milk production are rather constant, which leads to regular and voluntary visits to the AMS.

Farmers with an AMS are often worried that the number of voluntary visits to the AMS will decrease with grazing, leading to less milk production (de Haan *et al.*, 2010). Experiences of the 'group AMS' showed that this is true in some situations, but not in general. It was shown that even when the number of voluntary visits to the AMS decreases by 0.20 cow<sup>-1</sup>

day<sup>-1</sup>, the total milk production did not decrease. The problem of voluntary visits can be solved by motivating animals to come to the AMS, e.g. by offering roughage inside which is low in digestibility, using concentrates, not pushing the animals in situations where this is not needed (optimal criteria for getting cows), optimal selection of animals, additional labour and more AMS capacity than strictly needed.

The infrastructure of the farm yard and the available grassland area are often not well suited for grazing in combination with automatic milking. The best solution is to introduce a grazing box, which selects cows that are allowed to graze based on milk production and the time passed since the last visit to the robot. Other solutions are: cattle grids, remote controls for fences and/or automated closing of fences, and additional roads on the farm for the animals. It is also an option to accept more work. Especially in situations of construction of new buildings, careful attention should be paid to the infrastructure of the farm to ensure optimal solutions. A good infrastructure is essential for effectively combining grazing and automatic milking.

Based on three years of on-farm participatory research, the 'group AMS' of Koe&Wij formulated advice for combining grazing and automatic milking:

- Relax and let the cow be the boss.
- Install a grazing box.
- Continuous grazing provides rest for cow and farmer.
- Use animal grids and remote control.
- Provide a ration with enough roughage low in digestibility in the stable to motivate the cows to come to the AMS.
- If the farmer is eager to graze, it is possible and profitable.

#### Conclusions

Throughout north-western Europe, grazing is practised less on farms with an automatic milking system (AMS) than on farms without an AMS. Farmers with an AMS have to overcome some difficulties when they want to graze their animals. However, where the infrastructure and the grazing management are optimised, grazing will lead to a positive economic result with less labour needed.

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## References

De Haan M.H.A., Philipsen A.P., Van den Pol-van Dasselaar A., Holshof G., Lenssinck F.A.J. and de Koning C.J.A.M. (2010) Better grazing opportunities with a mobile milking robot. *Grassland Science in Europe* 15, 178–180.

Parsons D.J. and Mottram T.T.F. (2000) An assessment of herd management aspects of robotic milking on UK dairy farms. In: Hogeveen E. and Meijering A. (eds) *Robotic Milking*, Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 212–220.

Van den Pol-van Dasselaar A., Vellinga T.V., Johansen A. and Kennedy E. (2008) To graze or not to graze, that's the question. *Grassland Science in Europe* 13, 706–716.

Wiktorsson H. and Spörndly E. (2002) Grazing: an animal welfare issue for automatic milking farms. In: *The First North American Conference*, Wageningen Academic Publishers, Wageningen, The Netherlands, VI32-VI45.

# Session 2

# Forage and product quality

# Forage safety and quality hazards

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# Abstract

This paper summarises the safety and quality hazards in forages used for animal feed. In contrast to concentrate feeds such as cereal grains and oilseeds, forages often comprise the entire plant and contaminants deposited on farmland end up in the feed. Thus air-borne transport and deposition of contaminants poses a serious threat in forage production. Accidental nuclear fallout causes an acute problem that can be handled by discarding the crop, but also creates a long-lasting problem of soil contamination. Dioxin and PCB deposition from combustion industries is a continuous, non-accidental pollutant process. Dioxin deposition in Europe has steadily decreased in recent decades as a result of environmental awareness and in some areas is now back to levels occurring at the beginning of the last century. The most serious safety threat in forage is probably natural toxins originating from fungal growth in the field or during storage. Endophytic and epiphytic fungal infections causing toxin formation may seriously impair forage quality. The field flora is a possible threat to animal health, but the toxins produced are normally not transferred to products such as meat and milk. Field-derived mycotoxins are particularly common in maize and smallgrain crops and to a lesser extent in grass crops. The storage flora, which includes fungi such as Aspergillus and Penicillium, represents a greater risk to animal and human health due to potential transfer of toxins into meat and milk. Storage-derived mycotoxins are produced in all types of improperly made silage.

Keywords: nuclear fallout, dioxin, deposition, endophytes, mycotoxins, silage

# Introduction

One feature that makes forage crops more susceptible to pollutants than other feed crops is that the complete plant is often consumed, whereas with grain, beans or oilseeds, for example, only the seed is used as animal feed. When the whole crop is consumed, all pollutants deposited on the field surface end up in the feed, while much remains in the harvest trash when only the seed is harvested. Aerial transport and deposition of radioactive elements such as caesium-137 (<sup>137</sup>Cs) from nuclear power plants or dioxin from sewage combustion are sources of contamination. The forage is either directly grazed by animals or harvested and preserved as hay or silage.

Another health risk associated with forages concerns naturally occurring microorganisms colonising the plant in the field or the harvested crop in storage. These microorganisms, most commonly fungi, can cause harm by producing toxins that are harmful to animals and man or by causing diseases in animals or humans by spores or direct colonisation.

This paper deals with forage quality when used as animal feed, in particular forage from grassland but also some other forages such as maize silage and other cereal whole crops where the total vegetation is harvested and used as fodder. The paper provides a broad summary of possible risks and hazards as regards content of chemicals and microorganisms with negative consequences for the animal itself or the consumer of animal products such as milk and meat. The possible risks for humans handling the feed are also dealt with to

some extent. Finally, some aspects of the interaction between quality parameters such as fungal contamination and nutritional quality parameters are discussed.

## Field-derived risks

## Airborne deposition

The accident in 1986 at the Chernobyl nuclear power plant, Ukraine, suddenly made the risk of radioactive deposition on grassland a reality over vast areas of Europe. Studies in previous decades had already investigated the airborne deposition of radioactive substances, their absorption into the animal and transmission to milk and meat (Ward et al., 1966). The deposition at that time originated mainly from testing of nuclear weapons, but the meltdown in Chernobyl led to heavy deposition of radioactive substances in many countries in Europe. Estimates of the total deposition of <sup>131</sup>I and <sup>137</sup>Cs show that about 3–5% of the total released from Chernobyl were deposited in Norway and less than 1% in the United Kingdom (Clark and Smith, 1988; Tveten et al., 1998). The fallout was closely linked to the rainfall pattern during the period when the plume from Chernobyl was passing over the country (McAulay and Moran, 1989). When deposition occurs on grassland, a fraction of the deposited material remains on the leafy parts of the crop and is consumed by the animals at grazing or as silage after harvesting. This part can theoretically be removed by harvesting the crop and not using it for feeding purposes. This was frequently done in Sweden in 1986. However, grazing cows directly consume contaminated grass and in Sweden the milk obtained from cows grazing <sup>137</sup>Cs-contaminated pastures was discarded for weeks. Another fraction of the deposited material is rinsed off the leaves into the soil by rain, leading to more long-term contamination of grassland.

In a study monitoring grass ecosystems in the years after the Chernobyl reactor accident (Papastefanou *et al.*, 2005), the authors observed a reduction in the radioactivity in *Gramineae* and *Poaceae* species from 122 to 6 mBq kg<sup>-1</sup> over a 16-year period, with a trend for decreasing <sup>137</sup>Cs at a removal half-rate of 40 months.

Another study quantified the influence of rainfall after foliage contamination by artificially contaminating grassland with caesium, strontium, barium and tellurium, followed by controlled rainfall of 8 or 30 mm h<sup>-1</sup> (Madoz-Escande *et al.*, 2005). Those authors concluded that the first rainfall much reduced the radionuclide concentration but after four rainfall events no further significant reduction was observed. They also observed a sharp accumulation of Cs and I in the soil close to the stem after a number of nights with dew, illustrating that the crop was being decontaminated even without rain.

When animals have ingested radionuclide-contaminated feeds, the concentration in the body tissues can be reduced by feeding a diet free from radionuclide contamination. This was common practice for many years after the Chernobyl fallout over Sweden and Norway before reindeer slaughter and in Norway also lamb slaughter. Those animals graze mountainous areas where the soil depth is low and turnover of Cs in the soil slow. A significant decrease in Cs content has been reported in lambs fed indoors on a Cs-free diet after accumulating high levels of Cs from grazing highly polluted pastures (Martin *et al.*, 1988).

In the years after the Chernobyl accident it was believed that the fallout would remain for a long time in grassland with thin soils, e.g. in many mountainous areas, while vertical transport would occur in deeper soils. Thus in the latter the effect would be attenuated and possibly also metabolised. However, a long-term vertical migration study in Bavaria showed that  $^{137}$ Cs had its maximum activity at a depth of 2–4 cm 7 years after deposition (Schimmack *et al.*, 1997). They concluded that 40% of the  $^{137}$ Cs activity originating from the Chernobyl fallout will still be active in the root zone of grassland 30 years after its deposition.

In a study investigating the transfer of <sup>131</sup>I and <sup>137</sup>Cs under natural conditions from Chernobyl fallout to milk and meat, the <sup>137</sup>Cs transfer coefficient for meat (<sup>137</sup>Cs intake per day/<sup>137</sup>Cs per kg product) was calculated to be 0.01 in dairy cow meat and 0.04 in meat from heifers and bulls (Voigt *et al.*, 1989). For milk, the transfer coefficient was 0.007 for <sup>131</sup>I and 0.003 for <sup>137</sup>Cs.

Radioactive fallout can be regarded as accidental and occasionally occurring, but with long-term consequences. However with the large number of nuclear power stations today compared with the 1980s and continents heavily loaded with nuclear arms, the risk of new accidents is always present and possibly increasing.

Another form of airborne deposition that is not accidental and which can have consequences for grassland is the spread of pollutants such as dioxins. Dioxins is sometimes used as a generic term for polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and coplanar-PCB. These are all formed during various combustion and industrial processes. They are typical low-dose contaminants from the increasingly industrialised society surrounding the grasslands all over Europe. As they are deposited in proportion to the surface area, grasslands which cover half the agricultural land in many countries in Europe are major recipients. A field study has shown that transfer of dioxins from air into fodder is about 35% of the atmospheric content, as determined by passive sampling of the grass (Schuler *et al.*, 1997).

Food is the major source of human exposure to dioxins and the fat fraction in milk and meat is an important vector in Europe, while seafood is the largest vector in Japan (Akihisa, 2001). Akihisa (2001) concluded that although forage is regarded as the major source of animal exposure to dioxins, this issue requires more investigation and that the most important approach is to encourage society to restrict the discharge of dioxins.

The call for reduced emissions of dioxins seems to have been met, at least in Europe. Since 1993, the Flemish Environmental Agency has monitored the deposition of dioxins in Belgium twice a year and has reported a significant decrease (Van Lieshout *et al.*, 2001). Emission reduction programmes and the closing down of major sources are the reasons behind the improvement. While high rates of deposition still occur near large ferrous foundries and other industries, annual average deposition is in the range 3.4–10 pg TEQ m<sup>-2</sup>day<sup>-1</sup> (Van Lieshout *et al.*, 2001).

A study in Germany investigated changes over time in the behaviour and spatial distribution of dioxin-like and indicator PCB in ambient air, deposition and plants (Rappolder *et al.*, 2007). The concentration in spruce and pine shoots was used to measure changes in plants over time. The study concluded that atmospheric contamination with PCDD and PCDF decreased by 75% from 1985 to 1997 at one of two urbanised areas in Germany (Warndt in the west) and by 40% from 1991 to 1997 at the other site (Duebener Heide in the east). However, from 1997 to 2004 the concentration stayed constant at a level of 1 ng WHO-TEQ kg dm<sup>-1</sup> at both locations.

In an even more retrospective study of archived herbage collected between 1861 and 1993 in south-east England, it was shown that the concentrations of PCDD and PCDF had changed considerably over time (Kjeller *et al.*, 1996). The concentrations peaked in the 1960s and have declined ever since. The most recent sample contained a combined PCDD and PCDF value that was only slightly above the concentration in pre-1900 samples. That study only dealt with samples from one site in the UK, but it is supported by many other studies providing evidence of the fact that improvements in technology, presumably in combustion activities (waste incineration and metal processing), have had a substantial impact over time.

#### Natural toxins – mycotoxins from endophytic fungi

Another contamination problem occurring with field-grown crops concerns fungal growth on the crop when it is still growing at the field and the possible harmful mycotoxins that fungi can produce. A special case of fungal infection is endophytic fungal colonisation where the fungus grows intercellularly in the stem and leaf of the crop. The most frequently found endophytes in grass are of the genera Neotyphodium, Epichloë and Acremonium (Ball et al., 1995; Clay, 1990; Latch et al., 1987; Saikkonen et al., 2000). The endophyte-grass interaction is considered to be symbiotic, since the fungus acquires nutrients and protection from the grass and the grass benefits from enhanced competitive abilities such as increased resistance to herbivores, pathogens and drought (Cheplick, 1998; Clay, 1990; Saikkonen et al., 2000). A number of studies have investigated Festuca and Lolium grasses with respect to the presence of endophytes in grasses collected from cultivated and natural grasslands. For example, Saikkonen et al. (2000) examined 14 grass species from natural populations in Finland and 97 agricultural cultivars of 13 grass species. In the natural grasses, 10 of the 14 species were found to host endophytes, with the highest proportions in *Festuca* spp. In the agricultural cultivars, however, endophytes were absent in 10 of the 13 species and were only found in Festuca pratensis and Lolium perenne. Latch et al. (1987) examined 64 collections of seeds from their centre of origin or from old pastures in Europe and found that 82% were infected with endophytes. They also examined 16 commercial cultivars and found, in line with Saikkonen in Finland, that only 25% of these were infected. Since the infection is present already in the seed and the occurrence in commercial seeds seems to be under 50%, it should be possible for the seed industry to use the presence or absence of endophytes in marketing. However, this distinction has still not been put into practice to a great extent in Europe (Persson, 2011). In contrast, the endophyte content in seeds are fully commercialised in Australia and New Zealand. A seed company in New Zealand offers eight different ryegrass epiphyte options including no endophyte, standard endophyte (not for feed), and a number of special endophytes not harmful to animals but providing a pasture protected against a number of insects like the argentine stem weevil and the adult black beetle (SpecialitySeed, 2012). The research behind was performed in New Zealand and USA where crop pest persistency was investigated by Bouton et al. (2001) and the effect on animal health by Hopkins et al. (2010).

Powell and Petroski (1993) described the alkaloid toxins in endophyte-infected grasses and concluded that the fungi grow entirely within the host plant and that each grass-fungus pair results in a unique mix of various alkaloids, of which some are highly toxic to herbivores. Endophytic toxins in grasses include ergot alkaloids in *Festuca arundinacea* and tremorgens (e.g. lolitrem B) in *Lolium perenne* (Cheeke, 1995). Imlach *et al.* (2008) described 'ryegrass staggers', a neurological condition of unknown origin that impairs motion function in livestock, as being caused by the same neurotoxin, the indole-diterpenoid compound lolitrem B. When animals graze pastures containing this compound, they develop uncontrollable tremors and perform uncoordinated movements. Brown *et al.* (2009) compared growing steers grazing *Festuca arundinacea* with high or low levels of infection by endophytes (*Neotyphodium coenophialum*) and found that the high level gave reduced serum enzymes, increased hepatic glucogenic enzymes and reduced liver and carcass mass.

During recent years attention has turned to neurological mycotoxicosis of horses and a connection to ryegrass staggers has been proposed. Johnstone *et al.* (2011) therefore performed a test to describe the clinical expression of lolitrem B intoxication in horses. All horses developed a variable degree of tremor and ataxia when exposed to lolitrem B, but no increased level of lolitrem B was found in the urine. Increased levels were seen in the blood but not strictly correlated to the symptoms. Limb swelling, heel lesions and serous nasal discharge were also observed in the most severely intoxicated horses.

#### Natural toxins – mycotoxins from epiphytic fungi

While the endophytic fungal flora in forages may not be so well known, the epiphytic fungi have been much more widely studied. Mycotoxins derived from fungi colonising the crop in the field, such as deoxynivalenol (DON) and zearalenone (ZEA) from *Fusarium* spp. and ochratoxin from *Aspergillus ochraceus*, are well known. Moulds and mycotoxins in the growing crop are a greater problem when it comes to crops such as maize and small-grain cereals. Driehuis (2011) recently summarised the major mycotoxigenic moulds found in silages in a review paper listing the most important moulds (Table 1). DON and ZEA were found in well over half the maize samples tested in the USA, Austria, Germany, the Netherlands and Denmark in published studies for the period 1989–2004. The findings of these toxins in samples of grass were much less frequent, closer to 10%. Findings of aflatoxin and ochratoxin were very low. Two of the references cited (Driehuis *et al.*, 2008a; 2008b) examined 169 and 290 feeds, respectively, in the Netherlands in 2004–2008. Aflatoxins, ochratoxin A, T2 and HT2 toxins, sterigmatomycin, fusarenon-X, diacetoxyscirpenol, ergotamine or penicillinic acid were not found in any case.

Mycotox- in group	Major toxins	Mould species	Crop	Field/ silage- derived
Aflatoxins	Aflatoxin $B_1$ ( $M_1$ ), $B_2$ , $G_1$ , $G_2$	Aspergillus flavus, A. para- siticus	Maize	Field
Trichothecenes	Type A: T <sub>2</sub> , diacetoxy- scirpenol Rtpe B: DON, niva- lenol	Fusarium langsethiae, F poae, F. sporotrichiodes F.graminearum, F. culmorum	Maize, Sg cereals <sup>1)</sup> Maize, Sg cereals Grass	Field Field
Fumonisins	Fumonisin B <sub>1</sub> , B <sub>2</sub>	F. vericillioides, F. prolifera- tum	Maize	Field
Resorcyclic acid Lactones	Zearalenone	F. graminearum, F. culmorum	Maize, Sg cereals Grass	Field
Ochratoxins	Ochratoxin A	A. ochraceus, Penicillium verrucosum	Sg cereals	Field
Alkaloids	Clavines, lysergic acid amide, ergotamine Lolitrem B, ergovaline	Claviceps purpurea Neotyphodium lolii, N coeno- phialum	Sg cereals Grass	Field Field
<i>P. roqueforti</i> toxins	Roquefortin C, my- cophenolic acid	P. roqueforti, P. paneum	All types of silages	Silage
<i>A. fumigatus</i> toxins	Gliotoxin, fumiga- clavines	A. fumigatus	All types of silages	Silage
M. ruber toxins	Monocaolin K, citrinin	Monascus ruber	All types of silages	Silage

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<sup>1)</sup> Sg cereals = small-grain cereals (wheat, triticale, rye, barley)

The recommended maximum mycotoxin levels set by the European Commission (2006) for cereal and cereal by-products including roughages are 8 mg kg<sup>-1</sup> and 2 mg kg<sup>-1</sup> air-dried matter for DON and ZEA, respectively. For maize and maize by-products including maize silage the recommended limit is 50% higher, 12 and 3 mg kg<sup>-1</sup> air-dried matter, respectively. The reason is not lower toxicity of DON and ZEA from maize, but acknowledgement of the reality that the prevalence in maize is both more common and higher.

It is noteworthy that Dreiehuis *et al.* (2008a; 2008b) did not detect any ochratoxin A in any sample, whereas 30% of wheat sampled in Germany, Denmark, Sweden, Norway and Finland and presented to the European Commission (2002) contained ochratoxin A.

It is reasonable to assume that the reason for the higher mycotoxin levels in silages of whole-crop maize and small-grain cereals than grass silage is that the whole crops grow for a longer time in the field. Most grassland crops are harvested after about 6 weeks of growth, while e.g. whole-crop maize is cultivated for 4–6 months before harvest. Small-grain wheat is grown for at least 3 months before harvest. This hypothesis is strengthened by Müller (2009), who harvested the primary growth of a grass sward in May, June or August, representing about 6, 10 or 14 weeks post start of growth and found that later harvest resulted in increased counts of yeasts, moulds and enterobacteria in the crop. The number of mould species was also highest in the herbage harvested in August.

The field-derived mycotoxins mostly occur in maize and small-grain whole crop biomass, feeds that are seldom fed fresh. Information about the stability of field-derived mycotoxins in the acid environment in silage is scarce and contradictory. ZEA is generally regarded as stable in silage (Garon *et al.*, 2006). DON and ochratoxin A are metabolised in the rumen, making cattle less sensitive than horses. ZEA is also metabolised in the rumen, but some of the metabolites are potentially harmful for ruminants. Besides the risk posed by mycotoxins to animal health, there is concern over transfer into milk and meat. Carry-over rates of DON, ZEA, ochratoxin A, fumonisin B<sub>1</sub> and ergovaline are, however, very low, reducing the risk to food security to a minimum (EuropeanCommission, 2006; Fink-Gremmels, 2008).

## Toxins in weeds

Another source of toxins is the toxin content in plants that are not wanted in the grasslands, the poisonous weeds. The presence, however, is different at almost all locations and no general review is therefore possible. The presence of poisonous weeds is more likely to occur in extensively managed grasslands since in most cases they are sensitive to repeated cuts. The natural grasslands are also more sensitive to systematic changes such as changes in stocking rate, climate change and in airborne deposition. In a review of potential changes in ecosystem responses to reduced oxidised nitrogen inputs, caused by the change from coal burning to gas and nuclear power, Stevens et al. (2011) pointed out that certain plants, e.g. the buttercup (Ranunculus repens, R. acris), is expected to be favoured. The buttercup can have poisonous effects on cattle and horses both when consumed and at frequent contact (Griess and Rech, 1997). Other, very powerful plants like the cowbane (*Cicuta virosa*), are in most cases controlled locally by eradication programs. Common ragwort (Senecio jacobaea) is an example of a poisonous plant that is also well known in Europe and often subjected to control schemes. In contrast to the common ragwort that is sensitive to frequent mowing, the closely related march ragwort (Senecio aquaticus) is more resistant to intensive management and is spreading in Europe (Suter and Lüscher, 2011). Senecio aquaticus is lower, 20-60 cm compared to S. jacobaea and grows under wet conditions. Both contain the pyrrolizidine alkaloids which are toxic to animals (Evans and Evans, 1949) that consume the plant but is also suspected to be transferred into milk and be a carcinogenic vector to humans.

## Storage-derived risks

When the forage is not grazed but harvested and stored, the quality risks are dominated by the potential development of fungi. Grass dried to hay readily develops moulds if not

adequately dried. The dust from mouldy hay is known to contain spores causing respiratory diseases in both animals and man. British researchers (Dixon et al., 1995; McGorum et al., 1998) reported significant increases in airborne endotoxins in management systems for horses using hay compared with other systems and proved that these were due to airborne toxins from the hay. Hay is used mostly for feeding horses and to some dairy cows with special, with special restrictions due to cheese manufacturing, while forage for all other cattle is principally conserved as silage. The main reason why certain cheese-producing dairies prohibit silage as a feed is the occurrence of the spore-forming bacteria Clostridium tyrobutyricum. The bacteria contaminate the crop during harvest and can easily grow in silage at DM contents below 30% and form spores. The bacteria or spores do not harm the animal ingesting the silage, but the heat-tolerant spores are transferred to the milk via direct contamination and can spoil cheese during the ripening process. By careful management at ensiling, it is possible to restrict the growth of Cl. tyrobutyricum in silage (Rammer, 1996). The frequency of silages with silage-derived moulds is very high. O'Brien et al. (2008) found fungal growth in round bale silages at 174 of 180 farms visited in Ireland. P. roqueforti was the dominant species and, as expected, there was a relationship between visible damage to the stretch polythene wrapping around the bales and the intensity of fungal growth. Even when mould is found in silage, toxins are not necessarily produced, as exemplified in the study by Driehuis et al. (2008b). In that case, the mycotoxins roquefortin C and mycophenolic acid were found at double the frequency in the top layer than in the centre of the same silos, although the infection rate with P. roqueforti was probably similar throughout the silo.

The review by Driehuis (2011) listed the major mycotoxigenic moulds originating from silage (Table 1). In contrast to the field flora, where maize and small-grain whole crop are more prone to mould infections, members of the storage flora such as Penicillium roqueforti, Aspergillus fumigatus and Monascus ruber are found in all types of silage. The ensiling process is supposed to be anaerobic and the detrimental moulds jeopardising the quality are more or less strictly aerobic. This means that mycotoxin prevalence in the silage is due to silage making not being correctly performed. The reason for aerobic conditions can be badly sealed plastic covers on bunker silos and air penetration into the silage mass is often facilitated by bad compaction, resulting in low density silage with high porosity. Another very common reason for air penetration is low rate of silage removal after opening, leaving the silo open for extended periods. Besides the risk of mould growth there is a risk of growth of the bacterium Listeria monoctogenes in the top layer of especially wet bunker silos with air penetration. In pH above 5.6 the Listeria monocytogenes, which is an environmental bacterium being both an animal pathogen and a plant saprophyte, can develop. Ingested by sheep or cattle the bacteria cause listeriosis with symptoms of uterine infections and encephalitis. In well preserved silage with low pH the bacteria is inhibited (Low and Donachie, 1997)

The mould species present in the crop in the field with full access to oxygen will not survive in the silage. Many studies, e.g. Müller (2009), reporting mould counts before and after ensiling show that there is no correlation between the mould count on filling the silo and that on emptying the silo after the ensiling process. The most common mould species found tend to be *P. roqueforti* and *P. paneum*, acid-tolerant moulds that can grow at very low oxygen levels with the production of roquefortin, mycophenolic acid, PR toxin, festuclavine, agroclavine and patulin (Nielsen *et al.*, 2006; O'Brien *et al.*, 2006). Another species, regarded as more dangerous, is *Aspergillus fumigatus*, since in addition to producing toxins that are ingested it also spreads spores with the capacity to invade the lungs of man and

animals, causing aspergillosis (Latgé, 1999). Growth of Aspergillus flavus and A. parasiticus in silages is limited compared with that of other mould species, but their presence has been reported. High levels of the mycotoxin aflatoxin produced by these species have been reported in badly managed silage with late harvested and already dead maize plants (Pereyra et al., 2008). Aflatoxin B, in silage is serious because in contrast to other mycotoxins in silage, the carry-over into milk (as M<sub>1</sub>) is significant, 1–6% (Fink-Gremmels, 2008). Most surveys of the hygiene quality of silages cover traditional silages at 20–40% dry matter. The hygiene quality is most often related to cattle health, or to transfer to meat and milk from cattle. During recent years the interest in using silage for feeding horses has rapidly grown in Europe (Bergero et al., 2002; Müller and Udén, 2007). The silage is commonly produced in round bales or small square bales with a DM content of above 50%. Due to its high DM content, it is often referred to as haylage. Producing haylage for horses involves a different concept than silage for dairy cows or beef cattle in terms of nutritional requirement, since horses often require lower energy and protein content than cattle in order to avoid obesity. Higher DM content is associated with higher pH and lower nutritional status, obtained by later harvested grass, introducing new challenges when it comes to conservation. As referred to earlier, Müller (2009) reported higher mould counts on grass harvested in August compared with June or July. However this was not the case after conservation, where the grass harvested in August had the lowest pH and longest aerobic stability after opening. The reason for this was probably a higher content of lactic acid bacteria in crop harvested in August. In an assessment of the hygiene quality of haylage fed to horses on 18 farms in Sweden (Müller et al., 2011), the potential threat of encountering dangerous moulds was confirmed, since Aspergillus fumigatus and Penicillium spp. were detected, although the overall mould count was low. The increasing use of haylage for horses has also turned attention to the risk of contamination of the crop with the soil bacteria *Clostridium botulinum*, since horses are regarded as being more sensitive to Botulinum type B than cattle (Gudmundsson, 1997). However, cases of botulism reported in the literature do not seem to be more frequently connected to silage than other circumstances.

# Conclusions

Air-borne transport and deposition of contaminants, e.g. radioactive compounds, dioxins and PCB, poses a serious threat in forage production. Moderate radioactive fallout has partly been handled by discarding the crop, but dioxin and PCB deposition requires long-term action to eliminate sources of emission. Another serious safety threat in forage is natural toxins originating from fungal growth in the field or during storage. Forage safety relies on agricultural practices as regards silages of maize, small-grain whole crops and other crops with a long cultivation season. Grassland silages are normally harvested after a shorter growing time and are not as exposed to growth of toxin-producing moulds in the field. An exception is endophytic fungi in grass. Silages of all crops run a risk of developing storage-derived growth of bacteria and toxin-producing moulds. All undesirable mould growth is a function of bad management allowing air into the anaerobic ensiling process. Silages with high dry matter content probably run a higher risk of developing dangerous fungal growth, since they have higher porosity and higher pH. An additional risk arises when silage of this type is used for horses, which do not have the advantage of a rumen to eliminate many of the mycotoxins.

## References

Akihisa Y. (2001) *The Potential Pathway of Dioxins in Grassland Husbandry*, Grassland Science. pp. 72–79. Ball O.J., Prestidge R.A. and Sprosen J.M. (1995) Interrelationships between Acremonium Iolii, Peramine, and Lolitrem B in Perennial Ryegrass. *Applied Environmental Microbiology* 61, 1527–1533. Bergero D., Peiretti P.G. and Cola E. (2002) Intake and apparent digestibility of perennial ryegrass haylages fed to ponies either at maintenance or at work. *Livestock Production Science* 77, 325–329.

Bouton J.H., Gates R.N. and Hoveland C.S. (2001) Selection for persistence in endophyte-free Kentucky 31 tall fescue. *Crop Science* 41, 1026–1028.

Brown K.R., Anderson G.A., Son K., Rentfrow G., Bush L.P., Klotz J.L., Strickland J.R., Boling J.A. and Matthews J.C. (2009) Growing steers grazing high versus low endophyte (Neotyphodium coenophialum)-infected tall fescue have reduced serum enzymes, increased hepatic glucogenic enzymes, and reduced liver and carcass mass. *Journal of Animal Science* 87, 748–760.

Candrian U., Zweifel U., Luthy J. and Schlatter C. (1991) Transfer of Orally-Administered [H-3] Seneciphylline into Cows Milk. *Journal of Agricultural and Food Chemistry* 39, 930–933.

Cheeke P.R. (1995) Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. *Journal of Animal Science* 73, 909–918.

Cheplick G.P. (1998) Genotypic variation in the regrowth of *Lolium perenne* following clipping: effects of nutrients and endophytic fungi. *Functional Ecology* 12, 176–184.

Clark M.J. and Smith F.B. (1988) Wet and dry deposition of Chernobyl releases. Nature 332, 245-249.

Clay K. (1990) Fungal endophytes of grasses. *Annual Review of Ecology and Systematics* 21, 275–297. Dixon P.M., Railton D.I. and McGorum B.C. (1995) Equine pulmonary disease: a case control study of 300 referred cases. Part 3: Ancillary diagnostic findings. *Equine Veterinary Journal* 27, 428–435.

Driehuis F. (2011) Occurrence of mycotoxins in silage. In: Proceedings of the II International Symposium on forage quality and conservation, 16-19th November 2011, Sao Pauo, Brazil.

Driehuis F., Spanjer M.C., Scholten J.M. and Te Giffel M.C. (2008a) Occurrence of mycotoxins in maize, grass and wheat silage for dairy cattle in the Netherlands. *Food Additives and Contaminants: Part B* 1, 41–50. Driehuis F., Spanjer M.C., Scholten J.M. and te Giffel M.C. (2008b) Occurrence of mycotoxins in feedstuffs of dairy cows and estimation of total dietary intakes. *Journal of Dairy Science* 91, 4261–4271.

European Commission. (2002) Assessment of dietary intake of ochratoxin A by the population of EU member states. Report of experts participating inTask 3.2.7. January 2002., in: D.-G. H. a. C. protection. (ed.), Brussels. European Commission. (2006) Commission recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. Evans W.C. and Evans E.T.R. (1949) Poisoning of farm animals by the marsh ragwort (*Senecio-aquaticus huds*). *Nature* 164, 30–31.

Fink-Gremmels J. (2008) Mycotoxins in cattle feeds and carry-over to dairy milk: A review. *Food Additives* & *Contaminants: Part A* 25, 172–180.

Garon D., Richard E., Sage L., Bouchart V., Pottier D. and Lebailly P. (2006) Mycoflora and Multimycotoxin Detection in Corn Silage: Experimental Study. *Journal of Agricultural and Food Chemistry* 54, 3479–3484. Griess D. and Rech J. (1997) Diagnosis of acute poisoning by Ranunculi (*Ranunculus acris* L and *Ficaria ranunculoides* L) in horses. *Revue De Medecine Veterinaire* 148, 55–59.

Gudmundsson S.H. (1997) Type B botulinum intoxication in horses: case report and literature review. *Equine Veterinary Education* 9, 156–159.

Hopkins A.A., Young C.A., Panaccione D.G., Simpson W.R., Mittal S. and Bouton J.H. (2010) Agronomic Performance and Lamb Health among Several Tall Fescue Novel Endophyte Combinations in the South-Central USA. *Crop Sci.* 50, 1552–1561.

Imlach W.L., Finch S.C., Dunlop J., Meredith A.L., Aldrich R.W. and Dalziel J.E. (2008) The Molecular Mechanism of "Ryegrass Staggers," a Neurological Disorder of K+ Channels. *Journal of Pharmacology and Experimental Therapeutics* 327, 657–664.

Johnstone L.K., Mayhew I.G. and Fletcher L.R. (2011) Clinical expression of lolitrem B (perennial ryegrass) intoxication in horses. *Equine Veterinary Journal*, no-no.

Kjeller L.O., Jones K.C., Johnston A.E. and Rappe C. (1996) Evidence for a Decline in Atmospheric Emissions of PCDD/Fs in the U.K. *Environmental Science & Technology* 30, 1398–1403.

Latch G.C.M., Potter L.R. and Tyler B.F. (1987) Incidence of endophytes in seeds from collections of Lolium and Festuca species. *Annals of Applied Biology* 111, 59–64.

Latgé J.-P. (1999) Aspergillus fumigatus and Aspergillosis. Clinical Microbiology Reviews 12, 310-350.

Low J.C. and Donachie W. (1997) A review of Listeria monocytogenes and listeriosis. *The Veterinary Journal* 153, 9–29.

Madoz-Escande C., Garcia-Sanchez L., Bonhomme T. and Morello M. (2005) Influence of rainfall characteristics on elimination of aerosols of cesium, strontium, barium and tellurium deposited on grassland. *Journal of Environmental Radioactivity* 84, 1–20. Martin C.J., Heaton B. and Robb J.D. (1988) Studies of 131I, 137Cs and 103Ru in milk, meat and vegetables in North East Scotland following the Chernobyl accident. *Journal of Environmental Radioactivity* 6, 247–259. McAulay I.R. and Moran D. (1989) Radiocaesium fallout in Ireland from the Chernobyl accident. *Journal of Radiological Protection* 9, 29.

McGorum B.C., Ellison J. and Cullen R.T. (1998) Total and respirable airborne dust endotoxin concentrations in three equine management systems. *Equine Veterinary Journal* 30, 430–434.

Müller C.E. (2009) Influence of harvest date of primary growth on microbial flora of grass herbages and haylage, and on fermentation and aerobic stability of haylage conserved in laboratory silos. *Grass and Forage Science* 64, 328–338.

Müller C.E. and Udén P. (2007) Preference of horses for grass conserved as hay, haylage or silage. *Animal Feed Science and Technology* 132, 66–78.

Müller C.E., Hultén C. and Gröndahl G. (2011) Assessment of hygienic quality of haylage fed to healthy horses. *Grass and Forage Science* 66, 453–463.

Nielsen K.F., Sumarah M.W., Frisvad J.C. and Miller J.D. (2006) Production of Metabolites from the Penicillium roqueforti Complex. *Journal of Agricultural and Food Chemistry* 54, 5216–5216.

O'Brien M., Nielsen K.F., O'Kiely P., Forristal P.D., Fuller H.T. and Frisvad J.C. (2006) Mycotoxins and Other Secondary Metabolites Produced in Vitro by Penicillium paneum Frisvad and Penicillium roqueforti Thom Isolated from Baled Grass Silage in Ireland. *Journal of Agricultural and Food Chemistry* 54, 9268–9276. O'Brien M., O'Kiely P., Forristal P.D. and Fuller H.T. (2008) Fungal contamination of big-bale grass silage on Irish farms: predominant mould and yeast species and features of bales and silage. *Grass and Forage Science* 63, 121–137.

Papastefanou C., Manolopoulou M., Stoulos S., Ioannidou A. and Gerasopoulos E. (2005) Cesium-137 in grass from Chernobyl fallout. *Journal of Environmental Radioactivity* 83, 253–257.

Pereyra M.L.G., Alonso V.A., Sager R., Morlaco M.B., Magnoli C.E., Astoreca A.L., Rosa C.A.R., Chiacchiera S.M., Dalcero A.M. and Cavaglieri L.R. (2008) Fungi and selected mycotoxins from pre- and postfermented corn silage. *Journal of Applied Microbiology* 104, 1034–1041.

Persson C. (2011), Personal communication. SW Seed. Malmö, Sweden. http://www.swseed.com.

Powell R.G. and Petroski R.J. (1993) Alkaloid toxins in endophyte-infected grasses. *Natural Toxins* 1, 163–170. Rammer C. (1996) Quality of grass silage infected with spores of Clostridium tyrobutyricum. *Grass and Forage Science* 51, 88–95.

Rappolder M., Schröter-Kermani C., Schädel S., Waller U. and Körner W. (2007) Temporal trends and spatial distribution of PCDD, PCDF, and PCB in pine and spruce shoots. *Chemosphere* 67, 1887–1896.

Saikkonen K., Ahlholm J., Helander M., Lehtimäki S. and Niemeläinen O. (2000) Endophytic fungi in wild and cultivated grasses in Finland. *Ecography* 23, 360–366.

Schimmack W., Flessa H. and Bunzl K. (1997) Vertical Migration of Chernobyl-Derived Radiocesium in Bavarian Grassland Soils. *Naturwissenschaften* 84, 204–207.

Schuler F., Schmid P. and Schlatter C. (1997) Transfer of Airborne Polychlorinated Dibenzo-p-dioxins and Dibenzofurans into Dairy Milk. *Journal of Agricultural and Food Chemistry* 45, 4162–4167. SpecialitySeed L. (2012) Endophyte Options.

Stevens C.J., Manning P., van den Berg L.J.L., de Graaf M.C.C., Wamelink G.W.W., Boxman A.W., Bleeker A., Vergeer P., Arroniz-Crespo M., Limpens J., Lamers L.P.M., Bobbink R. and Dorland E. (2011) Ecosystem responses to reduced and oxidised nitrogen inputs in European terrestrial habitats. *Environmental Pollution* 159, 665–676.

Suter M. and Lüscher A. (2011) Measures for the control of *Senecio aquaticus* in managed grassland. *Weed Research* 51, 601–611.

Tveten U., Brynildsen L.I., Amundsen I. and Bergan T.D.S. (1998) Economic consequences of the chernobyl accident in Norway in the decade 1986–1995. *Journal of Environmental Radioactivity* 41, 233–255. Van Lieshout L., Desmedt M., Roekens E., De Fré R., Van Cleuvenbergen R. and Wevers M. (2001) Deposition of dioxins in Flanders (Belgium) and a proposition for guide values. *Atmospheric Environment* 35, Supplement 1, S83–S90.

Ward G., Johnson J. and Wilson D. (1966) Deposition of fallout cesium 137 on forage and transfer to milk. *Public Health Reports* 81, 639–646.

Voigt G., Pröhl G., Müller H., Bauer T., Lindner J.P., Probstmeier G. and Röhrmoser G. (1989) Determination of the transfer of cesium and iodine from feed into domestic animals. *Science of The Total Environment* 85, 329–338.

# Progress in forage-based strategies to improve the fatty acid composition of beef

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## Abstract

Nutritional properties of foods are increasingly important factors to consumers. This paper is focused on fatty acid composition of beef and in particular n-3 polyunsaturated fatty acids (PUFA). Exploitation of forage based systems as a route to the production of beef with enhanced product quality characteristics merits further pursuit. Feeding n-3 rich forage increases linolenic acid (18:3n-3) and the longer chain PUFA (EPA, 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) in beef lipids. Species-rich pastures and organic systems can influence fatty acid in beef lipids. The range of factors influencing fatty acids in forages such as genotype, conservation method, fertilizer regime are discussed as are opportunities to maximise delivery of n-3 PUFA from forage. Increased focus on breeding for lipids in perennial ryegrass and new knowledge of genes involved in regulating lipid composition is highlighted. Lipolysis and biohydrogenation of dietary PUFA by ruminal micro-organisms is a major limitation to our ability to beneficially enhance the fatty acid composition of beef lipids. Recent progress in understanding the microbial species involved is reviewed along with studies investigating the fate of lipid-rich chloroplasts in the rumen. The levels of n-3 PUFA achieved in beef lipids is assessed relative to those set by the European Food Safety Authority for a food product to be labelled as 'a source of' or 'high in' *n*-3 PUFA. Currently, it is challenging for forage-fed beef to achieve the necessary levels.

Keywords: beef, forage, lipids; rumen,; chloroplast, fatty acid

# Introduction

Consumers are increasingly aware of the relationships between their diet, health and wellbeing and this is leading to preference for foods which are healthier and more nutritous (Verbeke et al., 2010). This has focused attention on individual foods and in particular animalderived foods have received considerable scrutiny over the last 15-20 years. Indeed ruminant products have received increased attention in very recent years due to concern about the environmental impact of ruminant production and health attributes of meat and milk. It has been suggested that consumers could contribute to a double win in reducing greenhouse gas emissions and improving their health by reducing consumption of ruminant-derived products (Garnett, 2009). In relation to nutritional properties of meat and milk, much attention has focused on amount of fat and improving the profile of fat by increasing the content of beneficial fatty acids (Givens, 2010; Doreau et al., 2011; Scollan et al., 2011). For ruminant foods and other foods much activity has focused on altering the lipid profile with a major aim of helping these fulfil the guidelines for fat intake by the World Health Organization (WHO, 2003). The WHO (2003) recommended that total fat, saturated fatty acids (SFA), n-6 polyunsaturated fatty acids (PUFA), n-3 PUFA and trans fatty acids should contribute < 15-30, < 10, < 5-8, < 1-2 and < 1% of total energy intake, respectively. Particular emphasis is placed on reducing the intake of SFA (considered to be associated with increased cholesterol) and increasing the intake of omega-3 PUFA. The beneficial effects of the longer chain n-3 PUFA, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA; 22:6*n*-3) in reducing the risk of cardiovascular disease, cancer and type-2 diabetes, and their critical roles for proper brain function, visual development in the foetus and for maintenance of neural and visual tissues throughout life are well recognised (Scollan *et al.*, 2006). Hence this has focused attention on increasing these fatty acids in foods. Numerous reviews have examined factors influencing the fatty acid composition of meat and milk (Scollan *et al.*, 2006; Givens and Gibb, 2008; Wood *et al.*, 2008; Maloney *et al.*, 2009; Shingfield *et al.*, 2010; Doreau *et al.* 2011). This short review paper examines recent progress in improving the fatty acid composition of beef with emphasis on omega-3 fatty acids from the plant through to muscle lipids is dependent primarily on (1) levels of omega-3 PUFA in the plant lipids and (2) lipolysis and biohydrogenation of plant lipids in the rumen. Hence attention is given to these two main factors and a summary is given on forage effects on composition of beef lipids. Effects of other aspects of product quality including colour shelf life and sensory attributes have been previously discussed by Scollan *et al.* (2006) and Wood *et al.* (2008).

## Forage lipids and factors influencing lipid composition

Plants are the primary source of n-3 fatty acids, both in terrestrial and marine ecosystems, as they have the unique ability to synthesise *de novo* 18:3n-3 which acts as the building block for the n-3 series of essential fatty acids, via elongation and desaturation pathways (Dewhurst *et al.*, 2006; Barceló-Coblijn and Murphy, 2009; Scollan *et al.*, 2006). This can have a considerable effect on the fatty acid composition of beef (Scollan *et al.*, 2006). Furthermore, concern over the sustainability of the use of fish and fish oils as sources of long chain PUFA coupled with the 'food vs. fuel' debate concerning the production of grains and oilseeds has provided impetus for exploiting the use of more sustainable sources of n-3 PUFA (Napier and Sayanova, 2005; Sunderasan, 2009). Thus, forage is a noteworthy option for improving fatty acid composition of beef as it provides a feed source which is cheaper, natural and environmentally sustainable (Scollan *et al.*, 2006; Maloney *et al.*, 2008). Plants produce the majority of the world's lipids, which function mainly as the basic components of cellular membranes, as an important energy store and play a role in acute biological activities, in both plants and most eukaryotic organisms (Harwood, 1996). Additionally in plants, they function as constituents of the plant surface layers.

Forages generally contain a small amount of total fatty acids of which, proportionally, only 0.05 is storage fat (triacylglycerol), 0.05 free fatty acids (FFA), 0.1 diacylglycerol and monoacylglycerol with the remaining 0.8 in the form of cell and organelle membrane lipid (Lee *et al.*, 2008). This membrane lipid portion includes phospholipids, galactolipids and sulpholipids (Thompson *et al.*, 1998). Phospholipids are generally associated with non-photosynthetic tissues such as roots and usually contain high proportions of the SFA 16:0 and MUFA 18:1*n*-9 (Hildebrand, 2012; Murata *et al.*, 1982; Yoshida *et al.*, 2007). Galactolipids, on the other hand, are most abundant in photosynthetically active leaves. They are the major lipid component of chloroplasts, particularly in the thylakoid membranes of the chloroplast lamellae, which are 50% lipid by weight (Hawke, 1973). The two major galactolipids are monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) which make up 40–50% and 25–30% of membrane lipid, respectively (Williams *et al.*, 1983; Leech and Walton, 1983). Chloroplast lipid contains high proportions of PUFA, of which 18:3*n*-3 is usually the predominant fatty acid; however some plants contain 16:3*n*-3 as the main fatty acid, such as spinach for example. These PUFA play an important structural

feature in photosynthesis and an essential role in the maintenance of the electron transport system (Williams *et al.*, 1983; Routaboul *et al.*, 2000).

Although forage has a low lipid content, ranging from 30 to 100 g kg<sup>-1</sup> of DM (Bauchart *et al.*, 1984), it can contribute greatly, and in some cases wholly, to the total amount of lipid ingested by the ruminant animal due to the large amount of dietary forage material consumed (Scollan *et al.*, 2008). Forage has a high PUFA content with around 50–75% 18:3*n*-3 and 6-20% 18:2*n*-6 and 16:0 (Dewhurst *et al.*, 2003; Hawke, 1973). A number of studies have highlighted a strong genetic correlation with regard to fatty acid composition, suggesting the potential for grass breeders to produce high lipid grasses (Dewhurst *et al.*, 2001). However large genotype x environment interactions have also been identified (Dewhurst *et al.*, 2003; Palladino *et al.*, 2009). Understanding, monitoring and manipulating grassland management will be key in the exploitation of high lipid grasses (Kingston-Smith and Thomas, 2003; Dewhurst *et al.*, 2001).

Fatty acid content and composition of forage has been shown to be affected by species, regrowth interval, leaf:stem ratio, season, growth stage and maturity, fertilizer regime, temperature and light intensity; which are discussed in more detail in subsequent sections (Palladino *et al.*, 2009; Hawke, 1973).

Species differences in terms of fatty acid content and composition have been reported by Dewhurst *et al.* (2001) and Boufaïed *et al.* (2003). In the case of Dewhurst *et al.* (2001), they reported that fatty acid profiles were distinct between species when compared under the same cut/management, however this distinction was less clear when species were compared across cuts. This may be explained by the highly significant genotype x cutting date interactions (P < 0.001). Table 1 presents typical fatty acid content of various grass and legume species.

Differences between cultivars is less consistent, with no variation found by Dewhurst *et al.* (2002) whilst Gilliland *et al.* (2002) and Elgersma *et al.* (2003b) found no variation in terms of total fatty acids but variation in proportion of 18:3*n*-3. It should be noted that these studies used a small number of cultivars which may have had low genetic variability (Palladino *et al.*, 2009).

Ongoing work by grass geneticists at Aberystwyth University has successfully identified sections of the genome, along with genomic markers, in perennial ryegrass which are linked with lipid synthesis. This work is likely to lead to new insights into genes regulating lipid synthesis including those involving in synthesis of 18:3n-3.

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			F	Fatty acids	(g kg <sup>-1</sup> of I	DM)	
		16:0	18:0	18:1 <i>n</i> -9	18:2 <i>n</i> -6	18:3 <i>n</i> -3	Total
	Annual ryegrass	4.71	0.45	1.12	3.99	17.89	29.14
	Meadow fescue	4.18	0.30	1.60	3.43	11.96	22.30
Grasses	Cocksfoot	3.94	0.38	0.78	3.73	11.54	21.07
	Smooth bromegrass	3.43	0.26	0.60	3.25	9.94	18.09
	Timothy	3.52	0.37	1.11	3.83	8.45	17.96
	White clover	4.98	0.83	1.45	4.64	16.04	28.95
Legumes	Red clover	4.06	0.81	1.73	4.99	9.24	21.54
-	Lucerne	4.09	0.76	1.01	3.75	6.55	16.77

Table 1. Fatty acid content of selected forage species averaged across cultivars (adapted from Boufaïed *et al.*, 2003)

The effect of season and stage of maturity on lipid content and composition appears to be coherent in the literature. High concentrations of fatty acids occur during primary regrowth, leafy regrowth and vegetative growth late in the season (Hawke, 1973; Bauchart *et al.*, 1984; Dewhurst *et al.*, 2001). Bauchart *et al.* (1984) found the lipid content of perennial ryegrass to be highest during early May and late September (spring and autumn) and at its lowest at the beginning of August (summer). This pattern has also been found with hybrid and Italian ryegrass by Dewhurst *et al.* (2001), however Elgersma *et al.* (2003a) found the highest concentrations of fatty acids during the summer. Boufaïed *et al.* (2003) and Palladino *et al.* (2009) reported that season significantly affected total fatty acids, 16:0, 16:1, 18:2*n*-6 and 18:3*n*-3 but not 12:0, 14:0, 18:0 or 18:1*n*-9.

Leaf:stem ratio is an important factor in relation to fatty acid content and composition, with 18:3*n*-3 proportion of young leafy grass ranging between 0.74 and 0.79 and older grass between 0.59 and 0.68 for perennial ryegrass harvested in autumn (Hawke, 1973). Fatty acid content declines from early to mid-season in line with reproductive stem growth, then increases again towards the end of the growth season as leaf content recovers. This effect of leaf proportion may explain the inconsistent results found by Elgersma *et al.* (2003a) as the cuts taken in the summer months were from leafy regrowth's whereas spring cuts contained 'stemmy' regrowth's, hence this cut resulted in the lowest fatty acid concentrations. Dewhurst *et al.* (2003) noted that Italian ryegrass remains 'stemmier' for longer than perennial ryegrass with hybrid ryegrass being intermediate.

Extended regrowth intervals have a negative impact on total fatty acids and concentration of individual fatty acids (Elgersma *et al.*, 2005). Dewhurst *et al.* (2001) found fatty acid losses of 17%, 25%, 34% and 45% for 18:0, 18:1*n*-9, 18:2*n*-6 and 18:3*n*-3, respectively, between 20d and 38d regrowth interval. In contrast to this, Elgersma *et al.* (2003b) found all fatty acids decreased with increased regrowth interval, apart from 18:0, 18:1n-9 and 18:2n-6. They also reported lower loss of 18:3n-3 (15–23%); however this may relate to the smaller difference in regrowth interval of 10d in their study compared to 18d in the study of Dewhurst *et al.* (2001). Elgersma *et al.* (2005) also reported the effects of regrowth interval on fatty acid proportions and found that the proportion of 16:1 and 18:3n-3 decreased while 16:0, 18:1n-9 and 18:2n-6 increased with a longer regrowth period. It seems that management which inhibits flowering can increase fatty acid content, as in the case of Bauchart et al (1984) with two early cuts and Dewhurst *et al.* (2002) with nine cuts per year.

The few recent studies which have investigated the effect of N on fatty acid content and composition report a positive effect of N fertilization on fatty acid concentration (Boufaïed et al., 2003; Elgersma et al., 2005; Witkowska et al., 2008; Salcedo, 2011). Boufaïed et al. (2003) found N fertilization increased total fatty acid as well as the concentrations of 14:0, 16:0, 16:1, 18:1n-9, 18:2n-6 and 18:3n-3 but had no effect on 12:0 and 18:0. Elgersma et al. (2005) and Salcedo (2011) also reported increases in total fatty acids, 16:0, 16:1, 18:1n-9, 18:2n-6 and 18:3n-3 with N fertilization, with no significant effect on proportions of individual fatty acids. Interestingly, the largest response to N fertilization in the study of Salcedo (2011) was with the intermediate treatment of 12 kg N ha<sup>-1</sup> month<sup>-1</sup> under grazing conditions, with increases of 7.5%, 19%, 10%, 7.3% and 23% of 16:0, 18:0, 18:1n-9, 18:2n-6 and 18:3*n*-3, respectively, when compared to the 0 kg N ha<sup>-1</sup> month<sup>-1</sup> treatment. Boufaïed et al. (2003) observed increases of 18%, 12% and 40% of 16:0, 18:2n-6 and 18:3n-3, respectively, with an overall increase of 26% total fatty acids when comparing 120 vs. 0 kg N ha<sup>-1</sup>. This effect was consistent over a wide range of vegetative and reproductive stages and across contrasting environmental conditions in the study by Witkowska et al. (2008). Elgersma et al. (2005) noted a strong linear relationship between actual CP concentration and 18:3n-3 concentration, in line with Boufaied et al. (2003). Since fatty acids are N-free the relationship between N fertilization and fatty acid concentration must be indirect. It is hypothesized that higher N availability stimulates grass DM production, therefore increasing leaf area and stimulating synthesis of metabolic compounds, including chlorophyll which is positively correlated with chloroplast lipid, and thus increasing fatty acid concentration (Witkowska *et al.*, 2008; Hawke, 1973). However further investigation is needed to understand this interaction and also whether N has a direct effect on fatty acid metabolism (Elgersma *et al.*, 2005; Witkowska *et al.*, 2008).

Pasture is richer in fatty acids, particularly 18:3*n*-3, when compared to conserved forage, as demonstrated by French *et al.* (2000). The extent of PUFA loss is dependent on method of conservation (Harfoot and Hazlewood, 1997). The main factors which may be responsible for fatty acid losses during conservation are microbial intervention during ensiling causing undesirable fermentations, oxidation during field wilting and lipolysis via primarily plant lipases (Lough and Anderson, 1973; Dawson *et al.*, 1977; Dewhurst *et al.*, 2003). These lipases are released in response to stress or natural senescence and cause the rapid release and degradation of membrane fatty acids, mainly 18:3*n*-3 and 18:2*n*-6.

The majority of research which has investigated fatty acid changes during forage conservation suggests that the main period where loss is most likely to occur is during field wilting. Boufaïed *et al.* (2003) found lower concentrations of all the major unsaturated fatty acids (16:1, 18:1*n*-9, 18:2*n*-6 and 18:3*n*-3) and total fatty acids in wilted grass compared to fresh grass. Elgersma *et al.* (2003b) and Dewhurst and King (1998) found comparable results, with marked decrease in total fatty acid and proportion of 18:3*n*-3, especially under extended wilt conditions (Dewhurst and King, 1998). Conversely Arvidsson *et al.* (2009) noted that wilting did not affect fatty acid proportions; however this difference may be due to differences in duration of wilting and/or differences in developmental stage. Interestingly, Chow *et al.* (2004) investigated changes in fatty acid content and composition during wilting and ensiling using 3 different cultivars of perennial ryegrass, namely Agri, Respect and Barnham. They found that wilting and ensiling had an effect on 18:3*n*-3 proportion comparable to that reported by Elgersma *et al.* (2003b) in two of the cultivars. However wilting and ensiling did not have a major effect on fatty acid composition in the Barnham cultivar.

Findings on the effects of silage additives on fatty acid content and composition conflict somewhat. Arvidsson *et al.* (2009) reported that additives had an effect on fermentation characteristics but did not affect fatty acid concentrations to a great extent. Dewhurst and King (1998) reported significant but relatively minor effects on levels and proportions on fatty acids in perennial ryegrass silage whereas Boufaïed *et al.* (2003) reported greater additive effects in silage and haylage from timothy. In their study, lactic acid bacteria lowered total fatty acids and 18:3*n*-3 concentrations while formic acid decreased 16:0, 18:2*n*-6, 18:3*n*-3 and free fatty acids. The dose rate of both lactic acid bacteria (LAB) and formic acid generally had no significant effect on fatty acid concentrations, apart from 18:2*n*-6 which was lower at the higher LAB dose rate of  $10^5$  CFU<sup>x</sup> g<sup>-1</sup> of fresh matter.

## Lipid metabolism in the rumen

Whilst forages are typically rich in PUFA, they are extensively biohydrogenated in the rumen which results in beef which is largely composed of monounsaturated and saturated fatty acids (14:0, 16:0, 18:0 and 18:1n-9) (Scollan *et al.*, 2006; Jenkins *et al.*, 2008, Kim *et al.*, 2009; Or-Rashid *et al.*, 2009; Lourenço *et al.*, 2010).

Before biohydrogenation occurs forage fatty acids have to be hydrolysed in order to be released in their free form by a process called lipolysis. Lipolysis can be accomplished somewhat by the plant's own lipases (Lee *et al.*, 2004) but is largely accomplished by microbial lipases (Harfoot, 1978). Subsequently the PUFA are rapidly hydrogenated by the rumen microbes resulting in the production of saturated fatty acids (principally stearic acid; 18:0) (Jenkins *et al.*, 2008; Lourenço *et al.*, 2010). This process also results in the formation of CLA and *trans* monoene intermediates, including *cis*-9, *trans*-11 CLA and Vaccenic acid (VA; *trans*-11 18:1). The majority of *cis*-9, *trans*-11 CLA in animal lipids is synthesized by delta-9 desaturase from ruminally derived VA (Scollan *et al.*, 2006), and hence factors influencing the production of ruminally derived VA have been well researched. Generally, research in this area has focused on developing nutritional strategies which influence lipolysis and biohydrogenation and identification of the microbiota involved with a view to either reduce stearic acid production and increase the production of *cis*-9, *trans*-11 CLA and VA.

Studies have characterised biohydrogenation under a wide range of dietary circumstances. The extent of biohydrogenation of dietary PUFA from a variety of different diets incluidng forages is typically 70–95 and 85–100% for 18:2*n*-6 and 18:3*n*-3, respectively (Jenkins *et al.*, 2008; Kim *et al.*, 2009; Or-Rashid *et al.*, 2009; Lourenço *et al.*, 2010). Much interest has been placed on understanding the impact of differing types of oil supplementation such as rapeseed, sunflower, linseed and/or fish oils. Oil supplementation in the diet results in an increased flow of *trans* 18:1 intermediates leaving the rumen and subsequent deposition in tissue lipids. Fish oils and marine algae are more effective than plant oils in increasing VA production in the rumen and its subsequent appearance in tissue lipids (i.e. Shingfield *et al.*, 2010). Other approaches have examined the application of ruminal protected lipids to help bypass lipolysis/biohydrogenation and achieve greater effects on tissue lipids (Jenkins *et al.*, 2008; Lourenço *et al.*, 2010).

In relation to forages much attention has been placed on the potential for plant secondary metabolites to reduce rumen lipolysis and/or biohydrogenation. Feeding red clover (*Trifolium pratense*) relative to grass silage (*Lolium spp.*) increases the content of PUFA in both milk and tissue lipids per unit on PUFA intake (Dewhurst *et al.*, 2003; Scollan *et al.*, 2008). This relates to the presence and activity of polyphenol oxidase (PPO) in red clover which is thought to protect membrane lipids against degradation in forage during both ensiling and in the rumen, resulting in reductions in the apparent biohydrogenation of PUFA in the rumen. The mechanisms underpinning these responses have been reviewed by Van Ranst *et al.*, (2010) and the PPO mechanism is considered to have high potential as a plant-based tool to help protect lipids from ruminal degradation. This is also an area of active interest to plant breeders as the opportunity to increase PPO activity in red clover and other grass species containing the active PPO as a mechanism to reduce biohydrogenation of PUFA.

The higher PUFA content in meat and milk of animals grazing 'species rich' grassland and alpine relative to improved lowland grass swards (see subsequent section) is considered to relate to inhibited or modified fatty acid metabolism in the rumen. The changes may be caused by plant secondary compounds which are associated with the numerous 'weed' species common in 'species-rich' grassland. Such compounds include polyphenol oxidase, discussed previously, and essential oils, saponins and catecholamines which inhibit lipases and possess anti-microbial properties (Lourenço *et al.*, 2010; Cabiddu *et al.*, 2010; Khiaosa-ard *et al.*, 2011). Tannins found in certain leguminous species inhibited biohydrogenation by affecting microbial communities (Vasta *et al.*, 2009; 2010).

#### Rumen microbiome-lipidome interactions

It is clear that in order to beneficially manipulate the fatty acid content of beef, we must increase our understanding of rumen microbiome-lipidome interactions. Previously the bacteria believed to have a predominant role in the biohydrogenation pathways have been categorized as Group A and B (Harfoot and Hazelwood, 1997): group A bacteria hydrogenate 18:2n-6 and 18:3n-3 to trans-11 18:1; in contrast, group B bacteria convert the same fatty acids to 18:0. Group A bacteria, are part of the *Butyrivibrio fibrisolvens* group, an ill-defined taxon that includes the genera Butyrivibrio and Pseudobutyrivibrio and the species Butyrivibrio proteoclasticus (Kopečný et al., 2003; Paillard et al., 2007; Moon et al., 2008). Group B bacteria (18:0 producers) form a tight grouping in which strains cluster together close to B. proteoclasticus (van de Vossenberg and Joblin, 2003; Wallace et al., 2006). Recently using studies whereby marine algae and fish oil interventions were used to manipulate biohydrogenation, it has become clear that the bacterial taxa involved in biohydrogenation are more diverse than previously thought (Boeckaert et al., 2008; Kim et al., 2008; Huws et al., 2010; 2011). Indeed, the biohydrogenating bacterial taxa identified recently include Prevotella, Lachnospiraceae incertae sedis and unclassified Bacteroidales, *Clostridiales* and *Ruminococcaceae*, with most species identified being as yet unculturable (Huws et al., 2011). These data and their implications suggest that targeted approaches against the biohydrogenating microbiota are highly unlikely.

Nonetheless, lipolysis may well be a more amenable target for manipulation of the fatty acid content of beef. There is very little information on ruminal microbial lipolysis, with most information dating back to the 1970's. Indeed, there are only a few culturable isolates with known lipolytic activity, and only two lipases have been retrieved so far from a rumen bacterial metagenome (Liu *et al.*, 2009). Recently we have also isolated twelve lipase/ esterase genes and two phospholipases from rumen fosmid-based metagenomic libraries, the sequences of which seem to stem from bacteria that we cannot as yet cultivate in the laboratory (Manuscript in preparation). We have also recently genome sequenced (draft genome) a culturable lipolytic rumen bacterium, *Anaerovibrio lipolytica* with subsequent annotation and biochemical characterization of three identified lipases. Thus our understanding of rumen lipolysis is increasing and we now need to evaluate whether manipulating lipolysis can affect biohydrogenation to any significant extent.

Irrespective of their potential biohydrogenation capabilities, microbial cells that flow to the duodenum from the rumen are an important source of fatty acids for absorption by the animal. Rumen bacteria are proportionally high in odd-chain and branched-chain SFA(Williams and Dinusson, 1973; Kim et al., 2005; Or-Rashid et al., 2007). There is also some evidence that the rumen bacteria incorporate linoleic acid (18:2n-6) (Hawke, 1971), but it is uncommon to find PUFA within bacteria (Goldfine, 1982). In contrast rumen protozoa are rich in beneficial PUFA, monounsaturated fatty acids and CLA (Emmanuel, 1974; Devillard et al., 2006; Huws et al., 2009; 2012), probably due to their ability to engulf chloroplasts (Huws et al., 2009; 2012;), which contain most of total plant 18:3n-3 within their thylakoid membranes (Hawke 1973). Intra-protozoal chloroplast lipid metabolism may also aid direct uptake of the main chloroplast fatty acids (16:0, 18:3n-3 and 18:2n-6) into the rumen protozoal membranes. It is also possible that co-localisation of chloroplasts with engulfed bacteria within food vacuoles can result in intra-protozoal lipolysis and biohydrogenation of the intra-protozoal chloroplasts, providing that co-localised bacteria have lipolytic and biohydrogenating capacities. This may contribute to the previously reported high proportional representation of CLA in rumen protozoa. In a recent study we evaluated
whether increasing intra-protozoal chloroplast resulted in increased throughput of PUFA to the duodenum by comparing flow of protozoa to the duodenum post feeding of a diet low in chloroplast (straw:concentrate) and high in chloroplast (fresh grass) (Huws *et al.*, 2012). This study demonstrated that feeding a fresh grass diet to the steers resulted in a higher protozoal chloroplast content but did not result in their increased contribution to PUFA present at the duodenum. The reason for this was that the protozoa were retained in the rumen on the grass diet for reasons which are currently unclear. Conclusively, we can increase the PUFA content of protozoa, the challenge ahead is to ensure adequate flow to the duodenum. In summary, manipulating lipolysis as well as increasing intra-protozoal chloroplast flow to the duodeum present many challenges and opportunities for improving the fatty acid composition of ruminant products.

#### Forage effects on the fatty acid composition of beef

The effects of forage feeding and other nutritional approaches on the fatty acid composition of beef have been regularly reviewed (Scollan *et al.*, 2006; Wood *et al.*, 2008; Maloney *et al.*, 2009; Doreau *et al.*, 2011). This section is focused largely on the effects of pasture feeding on *n*-3 PUFA and how the levels achieved compare with guidelines from the European Food Safety Authority (EFSA) in relations to claims which may be made.

The positive effects of feeding n-3 rich pasture on beef lipids are well acknowledged. Pasture feeding increases 18:3n-3 and also the longer chain PUFA EPA and DHA (Table 2; i.e. Warren et al., 2008). Generally levels of n-3 PUFA achieved are higher on fresh v. conserved forage, and increase with amount of pasture offered and length of time on pasture (Scollan et al., 2006). Many recent studies have demonstrated the impact of grazing botanically-diverse pastures and/or organic systems on fatty acid composition of milk and meat (for reviews see Dewhurst et al., 2006; Maloney et al., 2008). The effect of biodiverse or organic systems on the fatty acid composition of meat and in particular for beef, is less well documented than that for milk and cheese. Fraser et al. (2009) noted lower levels of intramuscular fat in steers fed on semi-natural rough grazing relative to improved upland pasture and the proportion of 16:0 was lower and 18:2n-6 and 18:3n-3 higher. Steinsheim et al. (2010) noted only very small differences in fatty acid composition in suckling calves grazing on cultivated pasture relative to free range mountain pastures. Costa et al. (2011) examined the fatty acid composition of veal in the Barrosã region in Portugal in farms located in lowland, ridge or mountains. The effects of farm location on fatty acids in longissimus dorsi was small (Table 2) but the levels of PUFA achieved, particularly EPA and DHA were high, relative to other studies reported in Table 2.

The European Food Safety Authority (EFSA) recently concluded on the level of long chain PUFA that a product must contain in order for it to be labelled as 'a source of' or 'high in' *n*-3 PUFA. They concluded that it should be based around the requirement for 250mg per day of EPA plus DHA or 2g per day of 18:3*n*-3, and would require 40 or 80mg EPA plus DHA per 100g to be labelled as 'a source of' or 'high in' *n*-3 PUFA respectively (European Food Safety Authority, 2009). Based on the studies presented in Table 2 and using 100 g<sup>-1</sup> day as an appropriate figure for daily beef consumption (Scollan *et al.*, 2006) then the beef from the forage-based studies summarised in Table 2 may provide ~ 0.71–34.4 mg d<sup>-1</sup> EPA and ~ 0.15–6.3 mg d<sup>-1</sup> DHA. It is interesting that the highest levels reported in Table 2 are for veal calves with a total EPA + DHA of ~ 41 mg d<sup>-1</sup> (Costa *et al.*, 2010). Thus, this is higher than the ~15% of the daily recommended intake for long chain PUFA required to be able to make a claim (250 mg d<sup>-1</sup>, as described above). For comparison, Dunne *et al.* (2011) when feeding heifers ruminally protected fish oil supplement compared to

	Sex	Total	18:2 <i>n</i> -6	18:3 <i>n</i> -3	EPA	DPA	DHA	Reference	
Concentrate	Steers	3667	210	8.1	2.6	8.6	0.6	Warren et	
Grass silage	Steers	2838	84.0	48.3	19.7	27.2	5.1	al., 2008*	
Concentrate	Bulls	2670	109.7	9.1	3.7	9.6	2.4	Nuernberg et	
Grass silage	Bulls	2300	99.4	38.4	13.3	18.4	3.5	al., 2005	
Pasture	Bulls	547	76.5	18.2	5.6	7.1	0.5		
1-month concentrate after pas-	Dulla	912	05.2	16.2	75	0.2	0.6	Aldoi at al	
ture	Dulls	015	95.5	10.5	1.5	9.5	0.0	Alual <i>et ut.</i> ,	
2-month concentrate after pas-	Dulla	1055	102.2	12.5	77	0.4	0.8	2011	
ture	Dulls	1055	105.5	12.3	1.1	9.4	0.8		
Grass silage	Steers	3081	73.2	22.5	12.9	3.6	2.5		
Grass/red clover silage (50:50	Steers	3630	02.8	3/11	13/	23.0	23	Scollan et	
DM basis)	SIEEIS	3039	92.0	34.1	15.4	23.9	2.3	al., 2008	
Red clover silage	Steers	3081	113.2	50.7	14.9	25.1	2.8		
Improved permanent pasture	Steers	2131	58.5	40.4	17.3	22.1	2.7	Fraser et al.,	
Semi-natural rough grazing	Steers	1601	57.7	32.5	18.3	19.2	2.3	2009	
Lowland (< 400m)	Veal	2660	162.4	25.3	35.7	23.2	6.3	Costa et al	
Ridge (400–700m)	Veal	3170	175.9	33.4	34.4	21.0	6.2	2011**	
Mountain (>700m)	Veal	150	5.97	1.0	0.71	0.50	0.15	2011	
Control	Heifers	2870	80.4	13.3	13.0	NA	3.4	Dunne et al.,	
Protected fish oil (275 g d <sup>-1</sup> )	Heifers	3890	82.0	27.9	52.3	NA	15.4	2011**	

Table 2. Effect of forage type and species rich pasture and a ruminally protected lipid supplement of the fatty acid composition of beef muscle (mg 100  $g^{-1}$  muscle)

<sup>\*</sup>data from Aberdeen Angus 19 month age group; <sup>\*\*</sup>data recalculated from intramuscular fat levels and proportions.

a control achieved levels of 52.3 vs. 13.0 and 15.4 vs. 3.4, for EPA and DHA, respectively. The maximal levels of EPA + DHA delivered in beef from the studies reported would be ~ 67 mg<sup>-1</sup> 100 g muscle (Dunne *et al.*, 2011). This is also higher than the ~ 15% of the daily recommended intake for long chain PUFA and as such this beef may be noted as a "source" of long chain PUFA. All the forage treatments reported in Table 2, fall well below the 15% of recommended daily intake for EPA and DHA. Similarly, all the treatments fall below the level of 2g 18:3*n*-3 per 100 g product. These aspects present considerable challenges to approach levels of PUFA for which claims may be made.

# Conclusions

Nutritional quality is an increasingly important factor contributing to food product quality. Much attention is given to increasing the content of *n*-3 PUFA in beef and other foods as increased consumption of long chain *n*-3 PUFA would be beneficial in improving health and well-being and reducing disease in man. Green forage rich in the 18:3*n*-3 is an important tool to increasing delivery of *n*-3 PUFA through the ruminant animal into meat (and milk). As the 18:3*n*-3 is the building block of the long chain *n*-3 PUFA (EPA and DHA) feeding forage can increasen these beneficial PUFA in meat. However, the levels of *n*-3 PUFA, 18:3*n*-3, EPA and DHA achieved by forage feeding fall below the level required to be able to claim that beef is either a "source" or "rich-in" *n*-3 PUFA. Hence, it is essential that the two main factors influencing the levels of *n*-3 PUFA in beef lipids are further addressed, namely (1) strategies to enhance levels of 18:3*n*-3 in forage and subsequent delivery into the animal and (2) increased ability to reduce lipolysis and/or biohydrogenation in the rumen. Recent progress in genetic control of lipids in perennial ryegrasses is likely to help significantly. Increased knowledge of the fate of the lipid rich chloroplast in the rumen represents a very exciting opportunity to deliver more beneficial *n*-3 PUFA from rumen through to the small intestine and hence to meat lipids.

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#### References

Aldai N., Dugan M.E.R., Kramer J.K.G., Martínez A., López-Campos O., Mantecón A.R. and Osoro K. (2011) Length of concentrate finishing affects the fatty acid composition of grass-fed and genetically lean beef: and emphasis on *trans*-18:1 and conjugated linoleic acid profiles. *Animal* 10, 1643–1652.

Arvidsson K., Gustavsson A.-M. and Martinsson K. (2009) Effects of conservation method on fatty acid composition of silage. *Animal Feed Science and Technology* 148, 241–252.

Barceló-Coblijn G. and Murphy E.J. (2009) Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: Benefits for human health and a role in maintaining tissue n-3 fatty acid levels. *Progress in Lipid Research* 48, 355–374.

Bauchart D., Verite R. and Remond B. (1984) Long-chain fatty acid digestion in lactating digestion in lactating cows fed fresh grass from spring to autumn. *Canadian Journal of Animal Science* 64, 330–331.

Boeckaert C., Vlaeminck B., Dijkstra J., Issa-Zacharia A., Van Nespen T., Van Straalen W. and Fievez V. (2008) Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition of dairy cows. *Journal of Dairy Science* 91, 4714–4727.

Boufaïed H., Chouinard P.Y., Tremblay G.F., Petit H.V., Michaud R. and Bélanger G. (2003) Fatty acids in forages. I. Factors affecting concentrations. *Canadian Journal of Animal Science* 83, 501–511.

Cabiddu A., Salis L., Tweed J.K.S. Molle G., Decandia M. and Lee M.R.F. (2010) The influence of plant polyphenols on lipolysis and biohydrogenation in dried forages at different phonological stages: in vitro study. *Journal of Science Food and Agriculture* 90, 829-835

Chow T.T., Fievez V., Ensberg M., Elgersma A. and De Smet S. (2004) Fatty acid content, composition and lipolysis during wilting and ensiling of perennial ryegrass (*Lolium perenne* L): preliminary findings. *Grassland Science in Europe* 9, 981–983.

Costa P., Costa A.F., Lopes P.A., Alfaia C.M., Bessa R.J.B., Roseiro L.C. and Prates J.A.M. (2011) Fatty acid composition, cholesterol and  $\alpha$ -tocopherol of Barrosã-PDO veal produced in farms located in lowlands, ridges and mountains. *Journal of Food Composition and Analysis* 24, 987–994.

Dawson R.M.C., Hemington N. and Hazlewood G.P. (1977) On the role of higher plant and microbial lipases in the ruminal hydrolysis of grass lipids. *British Journal of Nutrition* 38, 225–232.

Devillard E., McIntosh F.M., Newbold C.J. and Wallace R.J. (2006) Rumen ciliate protozoa contain high concentrations of conjugated linoleic acids and vaccenic acid, yet do not hydrogenate linoleic acid or desaturate stearic acid. *British Journal of Nutrition* 96, 697–704.

Dewhurst R.J. and King P.J. (1998) Effects of extended wilting, shading and chemical additives on the fatty acids in laboratory grass silages. *Grass and Forage Science* 53, 219–224.

Dewhurst R.J., Moorby J.M., Scollan N.D., Tweed J.K.S. and Humphreys M.O. (2002) Effects of a staygreen trait on the concentrations and stability of fatty acids in perennial ryegrass. *Grass and Forage Science* 57, 360–366.

Dewhurst R.J., Scollan N.D., Lee M.R.F., Ougham H.J. and Humphreys M.O. (2003) Forage breeding and management to increase the beneficial fatty acid content of ruminant products. *Proceedings of the Nutrition Society* 62, 329–336.

Dewhurst R.J., Scollan N.D., Youell S.J., Tweed J.K.S. and Humphreys M.O. (2001). Influence of species, cutting date and cutting interval on the fatty acid composition of grasses. *Grass and Forage Science* 56, 68–74.

Dewhurst R.J., Shingfield K.J., Lee M.R.F. and Scollan, N.D. (2006) Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology* 131, 168–206.

Doreau M., Bauchart D. and Chilliard Y. (2010) Enhancing fatty acid composition of milk and meat through animal feeding. *Animal Production Science* 51, 19–29.

Dunne P., Rogalski J., Chilos S., Monahan F.J., Kenny D.A. and Moloney A.P. (2011) Long chain *n*-3 polyunsaturated fatty acid concentration and colour and lipid stability of muscle from heifers offered a ruminally protected fish oil supplement. *Journal of Agricultural & Food Chemistry* 59, 5015-5025. Elgersma A., Ellen G., Horst H., Muuse B.G., Boer H. and Tamminga S. (2003a) Influence of cultivar and cutting date on the fatty acid composition of perennial ryegrass (Lolium perenne L.). *Grass and Forage Science* 58, 323–331.

Elgersma A., Ellen G., van der Horst H., Muuse B., Boer H. and Tamminga S. (2003b) Comparison of the fatty acid composition of fresh and ensiled perennial ryegrass (Lolium perenne L.), affected by cultivar and regrowth interval. *Animal Feed Science and Technology* 108, 191–205.

Elgersma A., Maudet P., Witkowska I.M. and Wever A.C. (2005) Effects of nitrogen fertilisation and regrowth period on fatty acid concentrations in perennial ryegrass (Lolium perenne L.). *Annals of Applied Biology* 147, 145–152.

European Food Safety Authority (2009) Scientific opinion of the panel on dietetic products, nutrition and allergies on a request from European Commission related to labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids. *The EFSA Journal* 1176 1–11

Fraser M.D., Davies D.A., Vale J.E., Nute G.R., Hallett K.G., Richardson R.I. and Wright I.A. (2009) Performance and meat quality of native and continental cross steers grazing improved upland pasture or seminatural rough grazing. *Livestock Science* 123, 70–82.

French P., Stanton C., Lawless F., O'Riordan E.G., Monahan F.J., Caffrey P.J. and Moloney A.P. (2000) Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. *Journal of Animal Science* 78, 2849 –2855.

Garnett T. (2009) Livestock-related greenhouse gas emissions: impacts and options for policy makers. *Environmental Science and Policy* 12, 491–503.

Gilliland T.J., Barrett P.D., Mann R.L., Agnew R.E. and Fearon A.M. (2002) Canopy morphology and nutritional quality traits as potential grazing value indicators for Lolium perenne varieties. *The Journal of Agricultural Science* 139, 257–273.

Givens D.I. and Gibbs R.A. (2008) Current intakes of EPA and DHA in European populations and the potential of animal-derived foods to increase them. *The Proceedings of the Nutrition Society* 67, 273–280. Goldfine H. (1982) Lipids of prokaryotes – structure and distribution. Current topics in membranes and transport. (eds S. Razin & S. Rottem), Academic Press, pp. 1–43.

Harfoot, C.G. (1978) Lipid metabolism in the rumen. Progress in Lipid Research 17, 21-54.

Harfoot C.G. and Hazlewood G.P. (1997) Lipid metabolism in the rumen. The Rumen microbial ecosystem (eds P.N. Hobson & C.S. Stewart), Springer, pp. 382–426.

Harwood J.L. (1996) Recent advances in the biosynthesis of plant fatty acids. *Biochimica Et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* 1301, 7–56.

Hawke J.C. (1971) The incorporation of long-chain fatty acids into lipids by rumen bacteria and the effect on biohydrogenation. *Biochimica et Biophysica Acta* (BBA) - *Lipids and Lipid Metabolism* 248, 167–170. Hawke J.C. (1973) Lipids. In Chemistry and Biochemistry of Herbage, G.W. Butler and R.W. Bailey, eds. (London: Acedemic Press), pp. 213–263.

Hildebrand, D. (2012) Production of unusual fatty acids in plants. http://lipidlibrary.aocs.org/plantbio/ unusualfa/index.htm [accessed on: 12/04/12]

Huws S.A., Kim E.J., Kingston-Smith A.H., Lee M.R.F., Muetzel S.M., Cookson A.R., Newbold C.J., Wallace R.J. and Scollan N.D. (2009) Rumen protozoa are rich in polyunsaturated fatty acids due to the ingestion of chloroplasts. *FEMS Microbiology Ecology* 69, 461–471.

Huws S.A., Kim E.J., Lee M.R.F., Scott M.B., Tweed J.K.S., Pinloche E., Wallace R.J. and Scollan N.D. (2011) As yet uncultured bacteria phylogenetically classified as Prevotella, Lachnospiraceae incertae sedis and unclassified Bacteroidales, Clostridiales and Ruminococcaceae may play a predominant role in ruminal biohydrogenation. *Environmental Microbiology* 13, 1500–1512.

Huws S.A., Lee M.R.F., Kingston-Smith A.H., Kim E.J., Scott M.B., Tweed J.K.S. and Scollan N.D. (2012) Ruminal protozoal contribution to the duodenal flow of fatty acids following feeding of steers on forages differing in chloroplast content. *British Journal of Nutrition*, in press.

Huws S.A., Lee M.R.F., Muetzel S.M., Scott M.B., Wallace R.J. and Scollan N.D. (2010) Forage type and fish oil cause shifts in rumen bacterial diversity. *FEMS Microbiology Ecology* 73, 396–407.

Jenkins T.C., Wallace R.J., Moate P.J. and Mosley E.E. (2008) Board-invited review: Recent Advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. *Journal of Animal Science* 86, 397–412.

Khiaosa-ard R., Oliva C.R., Kreuzer M. and Leiber F. (2011) Influence of alpine forage either employed as donor cow's feed or as incubation substrate on in vitro ruminal fatty acid biohydrogenation. *Livestock Science* 140, 80–87.

Kim E.J., Huws S.A., Lee M.R.F. and Scollan N.D. (2009) Dietary transformation of lipid in the rumen microbial ecosystem. *Asian-Australian Journal of Animal Sciences* 22, 1341–1350.

Kim E.J., Huws S.A., Lee M.R.F., Wood J.D., Muetzel S.M., Wallace R.J. and Scollan N.D. (2008) Fish oil increases the duodenal flow of long chain polyunsaturated fatty acids and trans-11 18:1 and decreases 18:0 in steers via changes in the rumen bacterial community. *Journal of Nutrition* 137, 2599–2607

Kim E.J., Sanderson R., Dhanoa M.S. and Dewhurst R.J. (2005) Fatty acid profiles associated with microbial colonization of freshly ingested grass and rumen biohydrogenation. *Journal of Dairy Science* 88, 3220–3230. Kingston-Smith A.H. and Thomas H.M. (2003). Strategies of plant breeding for improved rumen function. *Annals of Applied Biology* 142, 13–24.

Kopečný J., Zorec M., Mrázek J., Kobayashi Y. and Marinšek-Logar R. (2003) Butyrivibrio Hungatei Sp. Nov. and Pseudobutyrivibrio Xylanivorans Sp. Nov., Butyrate-Producing Bacteria from the Rumen. International *Journal of Systematic and Evolutionary Microbiology* 53, 201–209.

Lee M.R.F., Scott M.B., Tweed J.K.S., Minchin F.R. and Davies D.R. (2008) Effects of polyphenol oxidase on lipolysis and proteolysis of red clover silage with and without a silage inoculant (Lactobacillus plantarum L54). *Animal Feed Science and Technology* 144, 125–136.

Lee M.R., Winters A.L., Scollan N.D., Dewhurst R.J., Theodorou M.K. and Minchin F.R. (2004) Plantmediated lipolysis and proteolysis in red clover with different polyphenol oxidase activities. *Journal of the Science of Food and Agriculture* 84, 1639–1645.

Leech R.M. and Walton C.A. (1983) Modification of fatty acid composition during chloroplast ontogeny and the effects on thylakoid appression and primary photochemistry. In: Proceedings of the Sixth Annual Symposium in Botany. Presented at the Biosynthesis and function of plant lipids, American Society of Plant Physiologists, University of California, Riverside.

Liu K., Wang J., Bu D., Zhao S., McSweeney C., Yu P. and Li D. (2009) Isolation and biochemical characterization of two lipases from a metagenomic library of China Holstein cow rumen. *Biochemical and Biophysical Research Communications* 385, 605–611.

Lough A.K. and Anderson L.J. (1973) Effect of ensilage on the lipids of pasture grasses. *Proceedings of the Nutrition Society* 32, 61–62.

Lourenço M., Ramos-Morales E. and Wallace R.J. (2010) The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. *Animal*, 4, 1008–1023.

Maloney A.P., Fievez V., Martin B., Nute G.R. and Richardson R.I. (2008) Botanically diverse forage-based rations for cattle: implications for product composition, product quality and consumer health. *Grassland Science in Europe*, 13, 361–374.

Moon C.D., Pacheco D.M., Kelly W.J., Leahy S.C., Li D., Kopečný J. and Attwood G.T. (2008) Reclassification of Clostridium proteoclasticum as Butyrivibrio proteoclasticus, a butyrate-producing ruminal bacterium. *International Journal of Systematic and Evolutionary Microbiology* 58, 2041–2045.

Murata N., Sato N., Takahashi N. and Hamazaki Y. (1982) Compositions and Positional Distributions of Fatty Acids in Phospholipids from Leaves of Chilling-Sensitive and Chilling-Resistant Plants. *Plant and Cell Physiology* 23, 1071–1079.

Napier J.A. and Sayanova O. (2005) The Production of Very-Long-Chain PUFA Biosynthesis in Transgenic Plants: Towards a Sustainable Source of Fish Oils. *Proceedings of the Nutrition Society* 64, 387–393.

Or-Rashid M.M., Odongo N.E. and McBride B.W. (2007) Fatty Acid Composition of Ruminal Bacteria and Protozoa, with Emphasis on Conjugated Linoleic Acid, Vaccenic Acid, and Odd-Chain and Branched-Chain Fatty Acids. *Journal of Animal Science* 85, 1228–1234.

Or-Rashid M., Wright T. and McBride B. (2009) Microbial fatty acid conversion within the rumen and the subsequent utilization of these fatty acids to improve the healthfulness of ruminant food products. *Applied Microbiology and Biotechnology* 84, 1033–1043.

Paillard D., McKain N., Chaudhary L., Walker N., Pizette F., Koppova I., McEwan N., Kopečný J., Vercoe P., Louis P. and Wallace R. (2007) Relation between phylogenetic position, lipid metabolism and butyrate production by different Butyrivibrio-like bacteria from the rumen. *Antonie van Leeuwenhoek* 91, 417–422.

Palladino R.A., O'Donovan M., Kennedy E., Murphy J.J., Boland T.M. and Kenny D.A. (2009) Fatty acid composition and nutritive value of twelve cultivars of perennial ryegrass. *Grass and Forage Science* 64, 219–226.

Routaboul J.-M., Fischer S.F. and Browse J. (2000) Trienoic Fatty Acids Are Required to Maintain Chloroplast Function at Low Temperatures. *Plant Physiology* 124, 1697–1705.

Salcedo G. (2011) Effects of the application of nitrogen on the fatty acid profile of grass in coastal zone meadows in Cantabria (Spain) used for pasture. *Grassland Science in Europe* 16, 88–90.

Scollan N., Hocquette J.-F., Nuernberg K., Dannenberger D., Richardson I. and Moloney, A. (2006) Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science* 74, 17–33.

Scollan N.D., Gibson K., Ball R. and Richardson I. (2008) Meat quality of Charolais steers: influence of feeding grass versus red clover silage during winter followed by finish off grass. *Proceedings of the British Society of Animal Science* p. 52.

Scollan N.D., Greenwood P.L., Newbold C.J., Yanez Ruiz D.R., Shingfield K.J., Wallace R.J. and Hocquette J.F. (2011) Future research priorities for animal production in a changing world. *Animal Production Science* 51, 1–5.

Shingfield K.J., Lee M.R.F., Humphries D.J., Scollan N.D., Toivonven V., Reynolds C.K. and Beever, D.E. (2010) Effect of incremental levels of fish oil in the diet on ruminal lipid metabolism in growing steers. *British Journal of Nutrition* 104, issue 1, 56–66

Steinsheim H., Høglind M., Havrevoll Ø., Saarem K., Lombnaes I.H., Steinheim G. and Svendsen A. (2010) Performance and meat quality of suckling calves grazing cultivated pasture or free range in mountain. *Livestock Science* 132, 87–97.

Sunderasan S. (2009) The food v. fuel debate: A nuanced view of incentive structures. *Renewable Energy* 34, 950–954.

Thompson J.E., Froese C.D., Madey E., Smith M.D. and Hong Y. (1998) Lipid metabolism during plant senescence. *Progress in Lipid Research* 37, 119–141.

Van Ranst G., Lee M.R.F. and Fievez V. (2010) Red clover polyphenol oxidase and lipid metabolism. *Animal* 5, 4, 512-521.

Vasta V., Makkar H.P.S., Mele M. and Priolo A. (2009) Ruminal biohydrogenation as affected by tannins in vitro. *British Journal of Nutrition* 102, 82–92.

Vasta V., Yanez-Ruiz D.R., Mele M., Serra A., Luciano G., Lanza M., Biondi L. and Priolo A. (2010) Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. *Applied Environmental Microbiology* 76, 2549–2555.

Verbeke W., Pérez-Cueto F.J.A., Barcellos M.D. de, Krystallis A. and Grunert K.G. (2010) European citizen and consumer attitudes and preferences regarding beef and pork. *Meat Science* 84, 284–292.

Van de Vossenberg J.L. and Joblin K.N. (2003) Biohydrogenation of C18 unsaturated fatty acids to stearic acid by a strain of Butyrivibrio hungatei from the bovine rumen. *Letters in Applied Microbiology* 37, 424–428. Wallace R.J., Chaudhary L.C., McKain N., McEwan N.R., Richardson A.J., Vercoe P.E., Walker N.D. and Paillard D. (2006) Clostridium proteoclasticum: a ruminal bacterium that forms stearic acid from linoleic acid. *FEMS Microbiology Letters* 265, 195–201.

Warren H.E., Scollan N.D., Enser M., Hughes S.I., Richardson R.I. and Wood J.D. (2008) Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. I: Animal performance, carcass quality and muscle fatty acid composition. *Meat Science* 78 256–269

WHO (2003) Diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO expert consultation. WHO technical report series 916, Geneva

Williams P.P. and Dinusson W.E. (1973) Amino Acid and Fatty Acid Composition of Bovine Ruminal Bacteria and Protozoa. *Journal of Animal Science* 36, 151–155.

Williams J.P., Khan M.U. and Mitchell K. (1983) Galactolipid biosynthesis in leaves of 16:3- and 18:3plants. In: Proceedings of the Sixth Annual Symposium in Botany. Presented at the Biosynthesis and function of plant lipids, American Society of Plant Physiologists, University of California, Riverside.

Witkowska I.M., Wever C., Gort G. and Elgersma A. (2008) Effects of nitrogen rate and regrowth interval on perennial ryegrass fatty acid content during the growing season. *Agronomy Journal* 100, 1371.

Wood J.D., Enser M., Fisher A.V., Nute G.R., Sheard P.R., Richardson R.I., Hughes S.I. and Whittington F.M. (2008) Fat deposition, fatty acid composition and meat quality: A review. *Meat Science* 78, 343–358.

Yoshida H., Tomiyama Y., Tanaka M. and Mizushina Y. (2007) Distribution of fatty acids in triacylglycerols and phospholipids from peas (Pisum sativum L.). *Journal of the Science of Food and Agriculture* 87, 2709–2714.

# Session 2.1.

# Meeting forage quality and animal requirements

# Grazing regimes and fertilisation rates: effects on dry matter yields, crude protein content and digestibility of meadows in the Northeast of Portugal

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#### Abstract

The aim of this study was to evaluate the effect of two grazing regimes: the usual for the area and late spring grazing, and three rates of N fertilisation, on dry-matter (DM) yields and nutritive value in three mountain meadows (*Anthemido-Cynosuretum cristati*, *Gaudinio-Agrostietum cristati* and *Bromo-Cynosuretum cristati*). The results showed that the lowest DM yield was obtained on *Bromo-Cynosuretum cristati* meadow. Dry matter yields increased significantly with N fertilisation in all meadows, independent of grazing regimes. The highest nutritive values occurred at the beginning of spring, and decreased during the year as a result of maturation. This effect was favoured by N fertilisation and it was independent of grazing regimes. Nutritive values were sufficient to meet the daily requirements of beef cattle during the study.

Keywords: grazing management, fertilisation, nutritive value

#### Introduction

The growing season in Mediterranean mountains limits the production of meadows to spring and autumn (Pires and Moreira, 2000). Nevertheless, meadows are grazed during the year, beyond the usual cut for hay in spring. In the Northeast of Portugal, the hay cut represents more than 50% of yearly DM yields (Pires *et al.*, 2000), even in summer irrigated meadows, but with relative low nutritive value. Since the hay cut cannot be taken much earlier than the usual date, because of rainfall occurrence, the only way to improve its nutritive value is to reduce the period of growth and to close up meadows later. In this situation, dry matter (DM) yields and nutritive value of meadows must be studied because when the hay yield is increased, its nutritional value decreases due to advancing maturity in plants (Vázquez Aldana *et al.*, 2000). On the other hand, the effect of N fertilisation on DM yield and nutritive value, both as a direct or indirect effect through the changes it induces in the relative proportion of legumes and grasses (Sun *et al.*, 2008) must be studied too. The objective of the present study was to evaluate the effects of two grazing regimes, and three rates of N fertilisation, on dry matter (DM) yields and nutritive value in three mountain meadows.

# Materials and methods

In 1998 and 1999, three meadows were studied in the North-east of Portugal: *Anthemido-Cynosuretum cristati* (M1), *Gaudinio-Agrostietum cristati* (M2) and *Bromo-Cynosuretum cristati* (M3). The treatments consisted of two grazing regimes: usual for the area (G) and a late spring grazing (LG) period with meadows closed up for hay three weeks later; and three rates of N fertilisation (N0-0; N1-75 and N2-150 kg ha<sup>-1</sup> year<sup>-1</sup>). The experimental design was a hierarchical completely randomised split-split plot, where meadows (M) were the main plots, and grazing regimes (G and LG) the subplots and N fertilisation (N) the sub-subplots. Three samples were harvested inside exclosure cages of 0.25 m × 0.25 m

within sub-plots, at the beginning of spring (March/April), at the hay cut (June), and at the end of autumn (November). Samples were dried to constant weight at 60°C (48 h). Drymatter yields and nutritive value (crude protein content (CP), and digestibility (IVOMD)) were determined. CP content was analysed after macrokjeldahl digestion and the IVOMD was determined according to Marten and Barnes (1980). General linear models procedure (SYS-TAT 12) was used for ANOVA, and the difference among means was detected by Tukey's HSD test at the 0.05 probability level. Multiple regressions for CP yields and metabolizable energy (ME) were carried out. These models were adjusted by stepwise regression for each meadow taking the grazing treatment (G-LG), and the nitrogen fertilization (N) as independent variables. Only the independent variables with P < 0.05 were retained in the model.

#### **Results and discussion**

Anthemido-Cynosuretum cristati (M1) had the highest DM yields in spring and autumn, whilst Agrostietum cristati (M2) had the highest DM yields in the hay cut (Figure 1). The high N rate (N2) combined with late spring grazing (LG) significantly increased DM yields in M1 in spring (Figure 1). In this harvest, DM yields increased significantly in all meadows (P < 0.001) and in the usual grazing (G) with fertilisation (N1 and N2). Additionally, DM yields significantly increased by the late spring grazing (LG) but only in M1 in hay cut and in M3 in autumn. In the no-fertiliser treatment (N0), the proportions of the annual DM yields harvested in the hay cut accounted for 66–78–50% in G, and 64–70–55% in LG for meadows M1-3, respectively. In the case of the high N-rate treatments (N2) the proportions were 64–70–55% (G) and 63–79–58% (LG) in M1-3, respectively.



Figure 1. DM yields, in the three harvests. Different letters indicate significant differences (P < 0.05) between N fertiliser treatments (N0-0; N1:-75 and N2-150 kg N ha<sup>-1</sup>), and grazing regimem (G: usual grazing, LG: late spring grazing) in the same meadow (M1-3), and in the same harvest

It is well documented that maturation causes changes in chemical composition resulting in a decline in CP content and IVOMD (Vázquez Aldana *et al.*, 2000). Our results showed that the lowest nutritive values (CP content and IVOMD) were obtained in the hay cut and in autumn in all meadows, independent of N rate and grazing regimes, but values were sufficient to meet the daily requirements of beef cattle (NRC, 1996). Additionally, there was a significant interaction of fertilisation × grazing for nutritive value in spring (P < 0.001 and P < 0.01 for CP content and IVOMD, respectively) whereby late spring grazing (LG) combined with high N rate (N2) significantly increased CP content (Figure 2). While IVOMD content tended to be lower in LG regimen, a significant difference was detected only in the N1 treatment (Figure 2). On the other hand, the highest nutritive value was achieved with the highest N rates (N2) in all meadows. It is know that N fertiliser greatly increases the CP content and herbage digestibility (Duru, 2003). A different effect of grazing regimes (G) was obtained on ME and CP yields (Table 1) in meadows M1 and M2.



Figure 2. CP content and IVOMD in the three harvests. Different letters indicate significant differences (P < 0.05) between grazing regimem (G: usual grazing, LG: late spring grazing) in the same N fertililisation treatments (N0-0; N1-75 and N2-150 kg N ha<sup>-1</sup>) and harvest

While ME and CP yields increased with LG regimen and N fertilisation in M1, the opposite response was obtained in M2. This different response could be explained by the fact that the proportion of grasses in M1 was favoured by G regimen (and reduced the presence of legumes and other species) whilst on M2 the late spring grazing increased the presence of grasses (data not shown). Additionally, N fertilisation favours the presence of annual grasses (Sun *et al.*, 2008), e.g. *Gaudinia fragilis* (L.) Beauv on M2, which contributed to a reduction in ME yields. Yields of ME and CP increased significantly with N fertilisation of M3 and this effect was independent of grazing.

Table 1. Adjusted models for CP yields and ME in hay cut in meadows M1-3 taking the independent variables: grazing: 0 (G), 21 (LG) and N:  $0-150 \text{ kg N ha}^{-1}$ . (n = 36)

S
9808
11011
2 10475
242

#### Conclusions

When changes are introduced in meadow management it is important to note the initial community botanical composition. In our case, the change in spring management was more suitable for *Anthemido-Cynosuretum cristati* meadow, and less suitable for *Gaudinio-Agrostietum cristati*. N fertiliser increased DM yields and nutritive value in all meadows.

#### References

Duru M. (2003) Effect of nitrogen fertiliser rates and defoliation regimes on the vertical structure and composition (crude protein content and digestibility) of a grass sward. *Journal of the Science of Food and Agriculture* 83, 1469–1479.

Marten G.C. and Barnes R.F. (1980) Prediction of energy digestibility of forage with in vitro rumen fermentation and fungal enzyme systems. In: Pidgen W.C., Balch C.C. and Graham M. (eds) *Standardization of Analytical Methodology for Feeds*, IDRC, Ottawa, pp. 61–71.

NRC. (1996) Nutrient Requirements of Beef Cattle. National Academy Press. Washington, DC, USA.

Pires J. and Moreira N. (2000) Meadow pastures: Biomass yield and nitrogen response in the Northeast of Portugal. 11th FAO Meeting of the FAO working group of Mountain Grasslands: Quality and valorisations of mountain animal products. Luz St Sauver, Francia, pp. 25.

Pires J.M., Trindade H., Martins V. and Moreira N. (2000) *Produção ao longo do ano de pastagens de montanha sob o efeito da adubação azotada e do prolongamento do pastoreio na Primavera*. I- Produção de matéria seca na zona de Vila Real. III Reunião Ibérica de Pastagens e Forragens, Portugal-España, pp. 301–308.

Vázquez Aldana B.R., García-Ciudad A., Pérez-Corona M.E. and García-Criado B. (2000) Nutritional quality of semi-arid grassland in western Spain over a 10-year period: changes in chemical composition of grasses, legumes and forbs. *Grass and Forage Science* 55, 209–220.

Sun X., Luo N., Longhurst B. and Luo J. (2008) Fertiliser Nitrogen and factors affecting pasture responses. *The Open Agriculture Journal* 2, 35–42.

# Fatty acid content and lipid fractions in herbs

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# Abstract

Experiments have shown a higher transfer efficiency of n-3 and n-6 fatty acids (FA) to milk when feeding herbs compared to feeding grass-clover. With the aim to gain more knowledge for this, the FA profile of ten single plant species and the incorporation of FA in lipid fractions were analysed. The ten species were: chicory (*Cichorium intybus*), ribwort plantain (*Plantago lanceolata*), salad burnet (*Sanguisorba minor*), birds-foot trefoil (*Lotus corniculatus*), white meliot (*Melilotus officinalis*), caraway (*Carum carvi*), alfalfa (*Medicago sativa*), chervil (*Anthriscus cerefolium*), white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*). FA content in single species deviated considerably, although the main FA were C18:3n-3 and C18:2n-6, with chervil being the exception with C18:1n-9 as main FA. The majority of FA were found in the phospholipid and sterol fraction, with C18:3n-3 and C18:2n-6 as the dominating FA.

Keywords: herbs, fatty acid, lipid fraction

# Introduction

Feeding biodiverse herbage to dairy cows is known to increase milk content of polyunsaturated fatty acids (PUFA) (Dewhurst et al., 2006). In a previous study, we also showed that feeding a mixture of fresh herbs without grass and clover to dairy cows, increased the content of n-3 and n-6 fatty acids (FA) in milk to a higher content than expected compared to feeding fresh grass-clover or a traditional total mixed ration (TMR) composed of silage and concentrate (Petersen et al., 2011). The herb feeding resulted in a twofold increase in transfer efficiency from feed to milk for n-3 FA compared to both grass-clover and TMR diets. Several theories have been proposed to explain the higher transfer efficiency from feed to milk for PUFA when feeding herbs; one being that herbs alter rumen lipid metabolism, so that PUFA escapes biohydrogenation. We hypothesized that the incorporation of FA in certain lipid fractions affect biohydrogenation of FA, as FA embedded in more complex matrixes could be less prone to biohydrogenation than FA found in more simple matrixes. Information on FA content in single herb species is still lacking, and an understanding of FA content in single herb species could help to describe the association between FA in herbages and FA profile of milk. Therefore we are currently working on characterizing FA profile and the incorporation of FA in lipid fractions in single herb species, and afterwards will look into whether the incorporation of FA in lipid fractions can be linked to the transfer efficiency from feed to milk, when feeding herbs to dairy cows. Some results on FA profile and incorporation of FA in lipid fractions in single herb and plant species are presented in this paper.

# Materials and methods

The plant material was freeze-dried samples from the mentioned feeding experiment Petersen *et al.* (2011). Extraction of total lipid and fatty acids was analysed as described by Jensen (2008). Separation of lipid fractions was done by solid-phase extraction (SPE) (Persson *et*  *al.*, 2007). Briefly, the extracted lipids were applied to aminopropyl silica columns under vacuum. The columns were washed with chloroform, and the chloroform phase containing sterols and triglycerides (TAG) was dried under  $N_2$  at 40°C and kept for later analysis. Phospholipids were eluted with a chloroform:methanol mixture under vacuum. The non-esterified fatty acids (NEFA) were eluted with a mixture of chloroform:methanol:acetic acid under vacuum. The dried eluate containing sterols and TAG was dissolved in heptane and applied to a set of new columns and washed with heptane under vacuum to elute sterols. TAG was eluted with a mixture of heptane:chloroform:etylacetate. For further details on chemical and statistical analysis of the diets, see Petersen *et al.* (2011).

#### **Results and discussion**

FA content in single species deviated considerably, and total FA ranged from 9 g kg<sup>-1</sup> DM in white clover to 44.7 g kg<sup>-1</sup> DM in chervil with average FA content around 12 g kg<sup>-1</sup> DM. Results on FA in single herb species are scarce, but in agreement with results from Clapham et al. (2005), who looked at FA content in traditional and novel species, the most abundant FA was C18:3n-3, followed by C18:2n-6 (Table 1), making up approximately 50 and 20 percent of total FA, respectively. Chervil deviated in FA profile compared to the other species, with C18:1n-9 constituting 47 percent of total FA, and C18:3n-3 only three percent of total FA. The results of the present study only represent a two-week period in late summer, and thus a longer sampling period is needed to take seasonal variation into account. We hypothesized that incorporation of FA in certain lipid fractions could be one way to protect PUFA from biohydrogenation in the rumen and, to our knowledge, studies on incorporation and distribution of FA in certain lipid fractions in herbs are currently not available. Although the samples in this study only represent FA content in a limited timeframe, the preliminary results give an indication of distribution of FA in lipid fractions in single herb species (Table 1). The majority of FA was found in the phospholipid fraction, typically around 55 percent. The second largest fraction of FA was seen in the sterol fraction with around 40 percent, while TAG only contributes with a small fraction of the total FA, approximately around five percent. Percentage FA contributing to the NEFA fraction was very low, and the only noticeable NEFA fraction was in perennial ryegrass, where it contributed with one percent of total FA. Like in the FA profile chervil again made an exception. In contrast to

Spacios	FA com	FA composition, g kg <sup>-1</sup> DM			I FA, g Lipid distribution, % of total FA				
species	C18:1n-9	C18:2n-6	C18:3n-3	kg <sup>-1</sup> DM	Phospholipids	sterols	TAG	NEFA	
Chicory	0.3°	2.1°	4.1	9.1 <sup>bc</sup>	70	26	5	0	
Plantain	0.5 <sup>bc</sup>	2.9 <sup>bc</sup>	5.2	11.3 <sup>bc</sup>	60	36	4	0	
Caraway	0.7 <sup>b</sup>	4.0 <sup>b</sup>	5.5	15.3 <sup>bc</sup>	50	44	6	0	
Birdsfoot trefoil	0.3°	2.1°	8.1	13.8 <sup>bc</sup>	56	40	4	0	
Salad burnet	$0.4^{bc}$	2.6°	6.3	12.5 <sup>bc</sup>	55	40	5	0	
Alfalfa	$0.4^{bc}$	1.8°	4.2	9.6 <sup>bc</sup>	50	49	1	0	
White meliot	0.5 <sup>bc</sup>	2.0°	6.3	12.6 <sup>bc</sup>	50	46	4	0	
Chervil	20.9ª	16.3ª	1.3	44.7ª	19	49	32	0	
White clover	0.5 <sup>bc</sup>	2.0 <sup>c</sup>	3.4	9.0°	65	32	3	0	
Perennial ryegrass	0.5 <sup>bc</sup>	2.5°	10.2	17.6 <sup>b</sup>	70	28	1	1	

Table 1. Fat	y acid compositio	n of single species	and incorporation of	of total fatty a	acids in lipid fractions
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<sup>a-c</sup> means within a column with different superscripts differ ( $P \le 0.05$ ).

the other species, only 19 percent of total FA was found in phospholipids and one-third of total FA was found in TAG. The phospholipids and sterols were typically made up of C18:3n-3 and C18:2n-6, while C18:1n-9 in chervil contributed noticeably higher to the FA in phospholipids compared to the other species (data not shown). TAG also mainly consisted of C18:3n-3, with chervil again being different with over 50 percent of FA in TAG being C18:1n-9 (data not shown). These preliminary results indicate a higher proportion of sterol FA in most herb species compared to white clover and perennial ryegrass, which on the other hand contains a higher proportion of phospholipids. However, further studies are needed in order to elucidate whether a high content of sterol FA is responsible for a lower biohydrogenation in the rumen and to what extent distribution of FA in lipid fractions is affected by factors like season and maturity of the plant.

#### Conclusions

Fatty acid composition of single herb species deviated considerably, with18:1n-9 being the dominant fatty acids in chervil compared to C18:3n-3 in the other species. Preliminary results on the incorporation of fatty acids in lipid fractions in herbs indicate a higher proportion of fatty acids in the sterols in herbs, whilst perennial ryegrass and white clover have a higher proportion of phospholipids, but further studies are needed to elucidate the importance of these results.

#### References

Clapham W.M., Foster J.G., Neel J.P.S. and Fedders J.M. (2005) Fatty acid composition of traditional and novel forages. *Journal of Agricultural and Food Chemistry* 53, 10068–10073.

Dewhurst R.J., Shingfield K.J., Lee M.R.F. and Scollan N.D. (2006) Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science of Technology* 131, 168–206.

Jensen S.K. (2008) Improved bligh and dyer extraction procedure. Lipid Technology 20, 280-281.

Persson E., Löfgren L., Hansson G., Abrahamsson B. and Lennerrnäs H. (2007) Simultaneous assessment of lipid classes and bile acids in human intestinal fluid by solid-phase extraction and HPLC methods. *Journal of Lipid Research* 48, 242–250

Petersen M.B., Soegaard K. and Jensen S.K. (2011) Herb feeding increases n-3 and n-6 fatty acids in cow milk. *Livestock Science* 141, 90–94.

# Fermentation characteristics, energy value and concentration of ß carotene in yarrow (*Achillea millefolium* L.) silage

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# Abstract

The aim of the study was to obtain information on the fermentation characteristics, nutritive value and  $\beta$  carotene concentration of varrow (Achillea millefolium L.) silage harvested in summer 2010. The unwilted and wilted varrow was ensiled in small-scale laboratory silos. Concentrations of net energy for lactation (NEL) and metabolizable energy (ME) were assessed on the basis of the amount of gas which was produced during the incubation of samples with rumen liquor in vitro. Silage from unwilted material (UW) had lower dry matter (DM) concentration than silage from wilted material (W) (278 g kg<sup>-1</sup> vs. 539 g kg<sup>-1</sup>). The concentrations of lactic acid, acetic acid and butyric acid in UW and W silages were 56.61 g kg<sup>-1</sup> DM, 9.06 g kg<sup>-1</sup> DM, 0.05 g kg<sup>-1</sup> DM; and 3.59 g kg<sup>-1</sup> DM, 1.96 g kg<sup>-1</sup> DM, 0.00 g kg<sup>-1</sup> DM, respectively. W was characterised by higher concentration of water soluble carbohydrates (WSC) (120.1 vs. 69 g kg<sup>-1</sup> DM), lower concentration of ammonia (21 vs. 78 g kg<sup>-1</sup> DM total N) and lower concentration of β-carotene (166.5 mg kg<sup>-1</sup>) than UW (237.1 mg kg<sup>-1</sup>). The energy values of both silages were similar. It was concluded that both silages were well fermented. Wilting of varrow prior to ensiling is favourable from the point of concentration of WSC and restriction of proteolysis during the fermentation process, whilst ensiling of unwilted material enhances  $\beta$  carotene preservation.

Keywords: yarrow, silage fermentation,  $\beta$  carotene, nutritive value

# Introduction

Yarrow (*Achillea millefolium* L.), a flowering plant from the family *Asteraceae*, is very competitive in grassland stands. It influences forage quality on permanent grasslands affected by frequent droughts and it is one of the poorest plant species in terms of water-soluble carbohydrate content (WSC) since it contains only 89 to 133 g WSC kg<sup>-1</sup> DM (Lukač *et al.*, 2010) or even less (67 g WSC kg<sup>-1</sup> DM) during the summer cut (Isselstein and Daniel, 1996). The objective of this experiment was to investigate the ensiling characteristics of yarrow, and to assess the impact of wilting on fermentation characteristics, nutritive value and  $\beta$  carotene concentration in yarrow silages.

# Materials and methods

Plants used in the present experiment were collected on a natural meadow near Murski Črnci (46°37' N; 16°06' E, 190 m a.s.l. in eastern Slovenia). During the summer cut, the unwilted (UW) and wilted (W) (8 hours) yarrow was ensiled at the flowering stage in small-scale (1L) laboratory silos in four replications. Silos were stored in darkness at  $20.7\pm4^{\circ}$ C and opened after exactly 183 days. Dried (60°C) and ground (1 mm particle size) samples were taken for determination of dry matter (DM) content (103°C), water soluble carbohydrates (Naumann and Bassler, 1976) and crude protein (Kjeldahl method). The fermentation quality of the silages was assessed by the following parameters: pH, ammonia nitrogen, volatile fatty acids and lactic acid analysed by gas chromatography (Holdemann and Moore,

1975). Net and metabolisable energy value was assessed on the basis of the gas volume that was produced during the 24 h incubation of samples with rumen liquor *in vitro*, using regression equations suggested by Menke and Steingass (1987). The ß carotene content was determined by liquid chromatography (EN 12823-2).

#### **Results and discussion**

Main characteristics of plant material used for ensiling are shown in Table 1. Wilted (W) and unwilted (UW) material contained sufficient amount of WSC for favourable fermentation. Crude protein (CP) concentration was similar in both samples. The NEL and ME concentration were relatively low, the main reason being maturity (flowering stage) at harvest, whereas concentration of  $\beta$  carotene in yarrow samples were comparable to the concentrations in the leaves of Italian ryegrass (*Lolium multiflorum* Lam., 196 mg kg<sup>-1</sup> DM) and alfalfa (*Medicago sativa* L., 186 mg kg<sup>-1</sup> DM) (Kalač, 1983).

Table 1. Water soluble carbohydrates (WSC), crude protein (CP), net energy for lactation (NEL), metabolisable energy (ME) and  $\beta$  carotene concentration in unwilted (UW) and wilted (W) yarrow samples measured at harvest

Sample -	WSC	WSC CP NEL ME			β carotene	
Sample	g kg-	<sup>1</sup> DM	MJ kg	-1 DM	mg kg⁻¹ DM	
UW yarrow unwilted	69.9	136.0	4.42	7.69	199.7	
W yarrow wilted	101.8	140.0	4.99	8.51	163.1	

When opened, all silages had a pleasant aromatic smell of yarrow and its essential oils, without any signs of deterioration. Despite less intensive fermentation, the pH of silages was low enough to prevent the growth of clostridia. After 183 days of fermentation the concentrations of fermentation products were lower in W than in UW (Table 2). Even in the UW the concentration of lactic acid was lower than expected in direct-cut grass silages. High DM content and consequently lower activity of lactic acid bacteria are the reasons for a higher concentrations of WSC in W silage, when compared to UW (Table 3). Despite fermentation, the concentrations of WSC in silages were similar (UW silage) or even higher (W silage) than in the parent material. It can be speculated that relatively high WSC concentrations in silages were due to the action of plant hemicellulases during the ensiling process. Based on concentration of butyric acid and ammonia nitrogen, the quality of W silage can be considered superior to UW silage.

Table 2. Dry matter (DM), pH and concentrations of ammonia nitrogen ( $NH_3-N$ ) and organic acids in silages after 183 days of fermentation

Sample –	DM	- nU	NH <sub>3</sub> –N	Lactic acid	Acetic acid	Butyric acid
	g kg <sup>-1</sup>	рп	g kg <sup>-1</sup> total N		g kg <sup>-1</sup> DM	
UW silage	278	4.3	78.1	56.61	9.06	0.19
W silage	538	5.1	21.4	3.59	1.95	0.00
Р	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.05

The W silage had a 30% lower  $\beta$  carotene concentration than UW silage (Table 3). The results are in agreement with the results obtained on fresh material (Table 1). For better  $\beta$  carotene preservation in silages we recommend ensiling of UW material. We have observed unexpected increase of  $\beta$  carotene concentration during the fermentation of UW yarrow, which might be due to inappropriate storage of fresh samples (-20°C). Carotenoids remain stable only if they are stored at -80°C (Tausz *et al.*, 2003).

Samula	WSC	СР	NEL	ME	β carotene		
Sample	Sample WSC CP NE   g kg <sup>-1</sup> DM M M M   e 69.1 143.7 4.6   120.1 140.0 4.5	MJ kg	g <sup>-1</sup> DM	mg kg <sup>-1</sup> DM			
UW silage	69.1	143.7	4.64	8.06	237.1		
W silage	120.1	140.0	4.58	7.93	166.5		
Р	< 0.001	ns	ns	ns	< 0.01		

Table 3. Concentrations of water soluble carbohydrate (WSC), crude protein (CP), net energy for lactation (NEL), metabolisable energy (ME) and  $\beta$ -carotene in unwilted (UW) and wilted (W) yarrow silages

ns, not significant.

#### Conclusions

Yarrow silages were pleasantly aromatic, without any signs of deterioration. The W silage was characterised by lower ammonia, acetic acid and butyric acid concentration and was therefore considered as slightly better than UW. W silage had better fermentation characteristics than UW silage, even though  $\beta$  carotene was preserved better in the case of ensiling of UW material. Due to the low levels of NEL and ME, we suggest that yarrow should be harvested earlier, during its vegetative stage.

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#### References

Blümmel M. and Ørskov E.R. (1993) Comparison of in vitro gas production and nylon bag degradability of roughages in predicting feed intake in cattle. *Animal Feed Science and Technology* 40, 109–119.

EN 12823-2:2000 Foodstuffs. Determination of vitamin A by high performance liquid chromatography. Measurement of  $\beta$  carotene, 13 pp.

Holdemann L.V. and Moore W.E.C. (1975) Anaerobe laboratory manual. 3rd Edition. VPI Anaerobe Laboratory, Blacksburg, 132 pp.

Isselstein J. and Daniel P. (1996) The ensilability of grassland forbs. *Grassland Science in Europe* 1, 451–455. Kalač P. (1983) Losses of beta–carotene in unwilted forage crops during silage-making and feeding. *Animal Feed Science and Technology* 9, 63–69.

Lukač B., Žnidaršič T., Verbič J., Verbič J. and Kramberger B. (2010) Hranilna vrednost nekaterih zeli s trajnega travinja. In: Čeh T. *et al.* (eds) 19. Mednarodno posvetovanje o prehrani domačih živali. *ZadravčeviErjavčevi dnevi*, Kmetijsko gozdarski zavod Murska Sobota, Radenci, 275–283.

Menke K.H. and Steingass H. (1987) Schätzung des energetischen Futterwerts aus der in vitro mit Pansensaft bestimten Gasbildung und der chemischen analyse. *Übersichten zur Tierernährung* 15, 59–94.

Naumann K. and Bassler R. (1976) Die chemische Untersuchung von Futtermitteln. *Methodenbuch*. Band 3, Verlag Neumann, Neudamm, 265 pp.

Tausz M., Wonisch A., Grill D., Morales D. and Jiménez M.S. (2003) Measuring antioxidants in tree species in the natural environment: from sampling to data evaluation. *Journal of Experimental Botany* 54(387), 1505–1510.

# Synergy between cocksfoot and red clover silages on voluntary intake and digestive processes in sheep

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# Abstract

Grass-legume associations are recognized for their agronomic and environmental interests. However, little is known regarding the possible interactions between the plants that can modulate, positively or negatively, intake and digestion in the animal. To characterize these associative effects, ten sheep were fed with five proportions of cocksfoot (*Dactylis glomerata*) and red clover (*Trifolium pratense*) silages (in percentage, 0:100; 25:75, 50:50, 75:25, 0:100) according a repeated  $5 \times 5$  Latin square design. Measurements were carried out on intake and digestive processes. Animals fed with mixtures of cocksfoot and red clover increased their voluntary feed intake compared to theoretical values calculated from the voluntary intake of silages fed alone (P < 0.001). The highest synergetic effect was observed for the 50:50 proportion, with an increase of 8% dry matter intake per kg of metabolic body weight. In contrast, no clear associative effect was observed on total tract digestibility and on enteric methane emissions. The positive associative effect on voluntary intake could be due to a greater motivation of animals to eat, rather than a synergy on digestive efficiency. Associations of cocksfoot and red clover silages could improve the animal performances in addition to the interest of grass-legume mixtures for the environment.

Keywords: grass legume mixture, silage, synergy, intake, methane, sheep

# Introduction

Grass-legume associations are known to provide agronomic and environmental interests. However, little is known regarding the possible interactions between the plants that can modulate, positively or negatively, intake and digestion in the animal. In some cases, values observed with a combination of forages differ from the balanced median values of its components considered individually (Niderkorn and Baumont (2009) for a review). These effects could be present in conserved forages as well as in fresh ones. The aim of this study was to investigate the associative effects between cocksfoot (*Dactylis glomerata*) and red clover (*Trifolium pratense*) silages on intake behaviour, DM digestibility and methane emissions in sheep.

# Materials and methods

Cocksfoot cv. Starly and red clover cv. Diadem silages were made in 2010 from pure swards sown at the INRA Clermont-Ferrand-Theix Centre (France). Their chemical compositions, in g per kg dry matter (DM), were: neutral detergent fibre (NDF): 461 and 401 g, acid detergent fibre (ADF): 257 and 246 g, acid detergent lignin (ADL): 31 and 50 g, crude protein (CP): 156 and 187 g, respectively. Five proportions of cocksfoot and red clover silages (in percentage, 0:100; 25:75, 50:50, 75:25, 0:100) were allocated to ten 1-year-old Texel sheep at maintenance fed *ad libitum* (10% refusals) according to a repeated  $5 \times 5$  Latin square design. The experiment was carried out during five experimental sequences, each comprising an 8-day adaptation period followed by a 6-day measurement period during which voluntary intake and intake behaviour (Baumont *et al.*, 1997), DM digestibility and

methane emissions using the SF<sub>6</sub> gas tracer technique (Martin *et al.*, 2011) were measured. Data were subjected to statistical analysis using the MIXED procedure of SAS<sup>®</sup> v.9.1 software. The cocksfoot-red clover proportion, the experimental period and their interaction were considered as fixed effects and sheep as a random effect, and the Tukey-Kramer test was used to compare means. Linear and quadratic contrasts were also tested to highlight potential associative effects.

#### **Results and discussion**

The proportion of red clover silage impacted very significantly on all the parameters of intake behaviour (P < 0.001) (Table 1). The DM intake increased when the proportion of red clover increased from 0 to 50%, and then tended to stabilize when this proportion increased to 100% (Figure 1A). As a consequence, a quadratic effect (P < 0.001) was observed on this parameter indicating a synergy between cocksfoot and red clover silages on DM intake. An increase of 8% DM intake per kg of metabolic body weight compared to the theoreti-

Table 1. Intake parameters, dry matter digestibility and methane emissions in sheep fed with different proportions of cocksfoot and red clover silages

		Red clover (%)					<i>P</i> -	Ortho	gonal asts <sup>2</sup>
Item <sup>1</sup>	0	25	50	75	100	DEM	value	Linear	Quad-
									ratic
DM intake, kg d <sup>-1</sup>	1.29°	1.45 <sup>b</sup>	1.56 <sup>a</sup>	1.59ª	1.56 <sup>a</sup>	0.043	< 0.001	***	***
DM intake per BW <sup>0,75</sup> , g kg <sup>-0.75</sup> d <sup>-1</sup>	60.4°	68.0 <sup>b</sup>	72.2ª	$74.0^{a}$	73.3ª	1.88	< 0.001	***	***
NDF intake, kg d <sup>-1</sup>	0.59°	0.65 <sup>ab</sup>	$0.68^{a}$	0.66 <sup>ab</sup>	0.63 <sup>b</sup>	0.024	< 0.001	*	***
Eating time, min d <sup>-1</sup>	252 <sup>d</sup>	287 <sup>bc</sup>	285°	306 <sup>ab</sup>	311 <sup>a</sup>	21.2	< 0.001	***	ns
Eating rate, g DM min <sup>-1</sup>	5.5 <sup>b</sup>	5.3 <sup>b</sup>	5.9ª	5.7 <sup>ab</sup>	5.3 <sup>b</sup>	0.67	< 0.001	ns	**
DM digestibility (%)	68.4ª	67.8 <sup>ab</sup>	67.3 <sup>ab</sup>	67.3 <sup>ab</sup>	65.6 <sup>b</sup>	0.77	0.015	**	ns
Digestible DM intake, kg d-1	0.88°	0.99 <sup>b</sup>	1.05 <sup>ab</sup>	$1.07^{a}$	1.03 <sup>ab</sup>	0.032	< 0.001	***	***
Methane, g d <sup>-1</sup>	25.1 <sup>b</sup>	27.0 <sup>ab</sup>	29.9ª	29.8ª	24.3 <sup>b</sup>	1.32	< 0.001	ns	***
Methane, g kg <sup>-1</sup> DM intake	20.0ª	19.0ª	19.6ª	19.1ª	16.1 <sup>b</sup>	1.06	0.001	***	ns

<sup>1</sup>DM: dry matter; NDF: Neutral Detergent Fiber; SEM: Standard Error of the Means.

<sup>2</sup>Orthogonal contrasts: ns: Not significant \* = P < 0.05; \*\* = P < 0.01; \*\*\* = P < 0.001.

<sup>a,b,c,d</sup> The values with a different letter are significantly different (P < 0.05).

cal value calculated from the forages fed alone was observed for the 50:50 proportion. It is generally considered that plant cell walls would limit intake by rumen filling, so that animals would regulate their intake at a relatively constant NDF intake level (Van Soest, 1982). However, we observed in this study a clear associative effect between cocksfoot and red clover silages on NDF intake (P < 0.001) with a maximum for the 50:50 proportion (0.68 kg d<sup>-1</sup>) (Figure 1B). In addition, we observed a similar synergy (P < 0.001) on the eating rate (Table 1) indicating a greater motivation to eat with the mixture in equal proportions. This synergy was not explained by a difference in digestion efficiency as the DM digestibility decreased linearly with the increase of red clover proportion in the diet without quadratic effect on this parameter. However, potential differences in the kinetics of digesta passage throughout the digestive tract and in the profile of fermentation products in silages may also play a role. Regarding the methane emissions expressed by unit of DM intake, no associative effect was observed but the emissions were clearly lower for pure red clover compared to other treatments (P < 0.001).



Figure 1. Synergic effect on daily voluntary dry matter (DM) intake (A) and neutral detergent fiber (NDF) intake (B) in sheep fed with different proportions of cocksfoot and red clover silages. The solid line represents observed values and the dotted line represents theoretical values calculated from pure forages. Bars indicate standard errors

#### Conclusions

A synergy between cocksfoot silage and red clover silage can be observed in sheep on the parameters of intake behaviour such as DM and NDF intake and eating rate. This synergy seems to be due to a greater motivation to eat, rather than to a more efficient digestion. Methane emissions are reduced when animals are fed with pure red clover silage.

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#### References

Baumont R., Jailler M. and Dulphy J.P. (1997) Dynamic of voluntary intake, feeding behaviour and rumen function in sheep fed three contrasting types of hay. *Annales de Zootechnie* 46, 231–244.

Martin C., Koolaard J., Rochette Y., Clark H., Jouany J.P. and Pinares-Patiño C.S. (2011) Effect of release rate of the SF<sub>6</sub> tracer on methane emission estimates based on ruminal and breath gas samples. *Animal* (in press). Niderkorn V. and Baumont R. (2009) Associative effects between forages on feed intake and digestion in ruminants. *Animal* 3, 951–960.

Van Soest P.J. (1982) Intake. In: *Nutritional ecology of the ruminant animal*, O & B Books, Inc., Corvallis, OR, USA, pp. 287–293.

# Influence of conservation method on fatty acid composition of herbages of a permanent grassland meadow

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#### Abstract

The main sources of variation in the fatty acid composition of fresh herbage are plant species, growth stage, temperature and light intensity. The conservation method can also influence the fatty acid composition. In an experiment, the fatty acids composition of fresh grass, three silages with dry matter contents of 20, 35 and 50%, and of barn-dried and field-dried hay were investigated. Samples were taken at harvest and after a storage period of 115 days. The samples were freeze-dried and the fatty acids were determined by gas chromatography. In addition, the nutrient contents were analysed.

With increasing amounts of pre-wilting, the individual and total fatty acids, such as  $\alpha$ -linolenic and linoleic acid, decreased, which is due to leaf shatter and oxidative losses.

Differences were also found after the storage period. For the three different silages, the concentration of different fatty acids increased during the storage period. The silage with the lowest dry matter content had the highest fermentation intensity, the highest sugar degradation and the highest concentration of fatty acids. Conservation as hay caused fewer changes with respect to fatty acids before and after the storage period.

Keywords: silages, hay, fatty acids, linolenic acid, linoleic acid

# Introduction

Wilting forages for the production of hay, and to a lesser extent prior to ensiling, decreases fatty acid concentration due to oxidative losses and leaf shatter (Dewhurst *et al.*, 2006). As the leaves contain higher concentrations of fat and fatty acids than the stems (Wyss, 2012), the leaf shatter contributes to the loss of fatty acids during the drying period in the field. The objective of this study was to investigate the fatty acid concentration during the process of silage and hay making, both in the field and during the storage period.

# Materials and methods

The fatty acids composition of fresh grass, three silages with dry matter contents of 20, 35 and 50%, and of barn-dried and field-dried hay were investigated. All forages were produced in Posieux (altitude 650 m a.s.l.). The forage was a third cut from a permanent meadow. It consisted of 73% grasses (mainly ryegrass), 9% legumes and 18% herbs. The silages were ensiled in laboratory silos of a capacity of 1.5 l. For the barn-dried hay, samples of 4 kg were put in nets and the nets were put in the forage of a conventional barn drying system. For the field-dried hay, the hay was stored in a wooden box in a dry place. There were three replications of all variants.

Samples were taken during the harvest process in the field and after a storage period of 115 days. The samples were freeze-dried and the fatty acids were determined by gas chromatography (Alves *et al.*, 2008). In addition, a part of the samples were dried at 60°C, milled, and the crude ash, crude protein, crude fibre and sugar contents were analysed. The data were analysed with SYSTAT 12 using one-way ANOVA.

#### **Results and discussion**

The crude protein content tended to decrease with increasing amounts of pre-wilting (Table 1). For the fibre contents (ADF and NDF) there was no direct influence of the extent of wilting. The silages with 35 and 50% DM had a good fermentation quality, but the silage with 20% DM contained butyric acid.

	DM	Ash	CP	ADF	NDF	pН
Silage 20% DM	197	130	158	311	470	4.5
Silage 35% DM	373	135	150	287	441	4.8
Silage 50% DM	519	115	160	299	486	6.1
Barn dried hay	904	98	134	274	473	_
Field dried hay	884	95	134	283	502	_
SEM	1.9	0.5	1.1	1.4	5.6	0.01
Significance	***	***	***	***	***	***

Table 1. Dry matter (DM) – and nutrient contents of the silages and the hays (g kg<sup>-1</sup> DM)

SEM standard error of mean; \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001; ns – non significant.

More extensive wilting generally reduced individual fatty acids and total fatty acids (TFA) (Table 2). These changes are due primarily to leaf shatter losses; high reductions of the different fatty acids during the wilting-process were also found by Dewhurst *et al.* (2006) and Shingfield *et al.* (2005). The  $\alpha$ -linolenic acid (C 18:3 c9c12c15) was the most dominant fatty acid. Their proportion in the total fatty acids (TFA) decreased from 58% to 50% with more extensive wilting. The linoleic (C18:2 c9,c12) and palmitic acid (C16:0) had proportionally a decline of between 12 and 16%.

						-	
Variants	C16:0	C16:1	C18-0	C18:1	C18:2	C18:3	TEA
variants	C10.0	t3	C10.0	c9	c9c12	c9c12c15	1171
Fresh grass	4.4	0.6	0.4	0.6	4.4	19.8	34.3
Grass 20% DM	4.5	0.5	0.3	0.7	4.8	18.9	33.9
Grass 35% DM	4.4	0.6	0.4	0.7	4.2	17.2	31.6
Grass 50% DM	4.2	0.5	0.3	0.6	3.8	15.1	28.7
Grass 80% DM	4.0	0.4	0.3	0.5	4.1	12.8	25.7
Grass 88% DM	4.1	0.5	0.3	0.5	3.5	13.4	25.8
SEM	0.09	0.04	0.01	0.03	0.21	0.74	1.04
Significance	*	ns	ns	***	*	***	***

Table 2. Fatty acid concentrations in the different forage prior to storage (g kg<sup>-1</sup> DM)

SEM standard error of mean; \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; ns – non significant.

Table 3. Fatty acid	concentrations	in the	different	forage at	fter storage	period (s	g kg <sup>-1</sup>	DM)
5				0	0	1		

Variants	C16:0	C16:1	C19.0	C18:1	C18:2	C18:3	TEA
	C10:0	t3	C18:0	c9	c9c12	c9c12c15	ІГА
Silage 20% DM	4.8	0.6	0.4	0.7	5.2	21.1	37.7
Silage 35% DM	4.6	0.6	0.4	0.7	4.8	19.5	34.9
Silage 50% DM	4.5	0.5	0.4	0.6	4.5	17.3	32.1
Barn dried hay	4.1	0.5	0.3	0.5	3.9	14.3	27.1
Field dried hay	3.9	0.5	0.3	0.5	3.7	14.0	26.8
SEM	0.12	0.02	0.01	0.01	0.16	0.63	1.20
Significance	**	**	***	***	***	***	***

SEM standard error of mean; \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; ns - non significant.

The fatty acid concentrations in the different forage after the storage period are shown in Table 3. The concentrations of individual and total fatty acids were higher in the silages than in the

wilted grass at ensiling. The silage with the lowest dry matter content had the highest fermentation intensity, the highest sugar degradation and the highest concentration of individual and total fatty acids. According to Boufaied *et al.* (2003) silage had higher concentrations of C16:0, C18:2, C18:3 and TFA than fresh grass. For the two hay variants, the values after the field and after the storage period were more similar in comparison to the silage variants. Figure 1 shows the strong relation between the DM-contents and the total fatty acids (TFA) after the field and storage period.



Figure 1. Relation between the DM-contents and the total fatty acids (TFA) after the field and storage period

#### Conclusions

The degree of pre-wilting strongly influenced the individual and total fatty acids in the forage. In the silages the individual and total fatty acids increased during the storage period. In the hay variants there were fewer changes concerning the fatty acids found during the storage period.

#### References

Alves S.P., Cabrita A.R.J., Fonseca A.J.M. and Bessa R.J.B. (2008) Improved method for fatty acid analysis in herbage based on direct transesterification followed by solid-phase extraction. *Journal of Chromatography* 1209, 212–219.

Boufaïed H., Chouinard P.Y., Tremblay G.F., Petit H.V., Michaud R. and Bélanger G. (2003) Fatty acids in forages. I. Factors affecting concentrations. *Canadian Journal of Animal Science* 83, 501–511.

Dewhurst R.J., Shingfield K.J., Lee M.R.F. and Scolan N.D. (2006) Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology* 131, 168–206.

Shingfield K.J., Salo-Väänänen P., Pahkala E., Toivonen V., Jaakkola S., Piironen V. and Huhtanen P. (2005) Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of milk. *Journal of Dairy Research* 72, 349–361.

Wyss U. (2012) Fatty acid composition of three different grassland species. *Grassland Science in Europe* 17 (this volume).

# Tannin extracts and their BSA precipitation ability at different pH values in vitro

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# Abstract

Tannins can form complexes with proteins in the rumen, increasing the amounts of protein bypass. These complexes are supposed to dissociate at low pH in the abomasum. Plant extracts containing tannins may be useful additives in dairy cattle nutrition. Results of a preliminary *in vitro* study are presented aiming to determine the ability of extracts from *Schinopsis lorentzii* (quebracho), *Acacia mearnsii* (mimosa), *Caesalpinia spinosa* (tara), and *Uncaria gambir* (gambier) to precipitate bovine serum albumin (BSA) at six pH values between 7 and 2 under standardized *in vitro* conditions.

Increasing amounts of extract were added to a BSA buffer solution. For each combination of extract type and pH value, precipitated protein was measured and data were fitted to a log – logistic equation culminating in a plateau.

Mimosa precipitated all BSA, independent of pH value, and achieved significantly higher plateaus than quebracho, except at pH 7. Tara plateaus were comparable to mimosa, except at pH 3 and 6. Gambier often did not achieve plateaus for the range of quantities of extract. The data suggest that precipitation ability of the extracts hardly changed with decreasing pH, except for quebracho. Only protein complexes with quebracho responded to pH change *in vitro* and may dissociate in the abomasum.

Keywords: condensed tannin, nitrogen-use efficiency, quebracho, mimosa, tara, gambier

# Introduction

It is supposed that condensed tannins form complexes with proteins, protecting them from ruminal degradation, and more feed protein is expected to pass the rumen undegraded (Barry and McNabb, 1999; Cassida *et al.*, 2000). These complexes are assumed to dissociate again in the low pH conditions of the abomasum (Jones and Mangan, 1977), making the protein available for the ruminant and thereby increasing nitrogen-use efficiency. Many legumes and other field herbs contain condensed tannins (CT), but have only comparatively low tannin content. Therefore, the objective of the project is to investigate the possible use of high concentrated tannin extracts as feed additives. In a preliminary *in vitro* study, different tannin extracts were tested for their protein precipitation ability at variable pH values.

# Materials and methods

CT were determined by butanol/HCl method (Terrill *et al.*, 1992); total phenols (TP) were determined by the folin-ciocalteu method (Singleton and Rossi, 1965). For analyses, tannin extracts of *Schinopsis lorentzii* (quebracho), *Acacia mearnsii* (mimosa), *Caesalpinia spinosa* (tara), and *Uncaria gambir* (gambier) were used *in vitro* to complex with bovine serum albumin (BSA) at six pH values between 7 and 2 under standardized conditions. Protein precipitation was then measured photometrically by a modulated method of Osborne and McNeill (2001). Samples of each tannin extract were weighed into 2 ml tubes in 1 mg increments between 0 to 10 mg. BSA was resolved at 1.5 mg ml<sup>-1</sup> in a sodium acetate solution. The solution was inserted to match 60 µg BSA per tube, the tube was then vortexed.

After 15 min resting at room temperature the tubes were centrifuged at 2400 g for 20 min. The produced protein pellet was resolved in 500  $\mu$ l solution for photometrical analyses by Bio-Rad Protein Assay (Bio-Rad Laboratories GmbH, Munich, Germany) at 595 nm (Biochrom Libra S32PC UV, Biochrom Ltd., Cambridge, UK). To avoid distortion of measurements by the extract's own protein, controls were performed without any BSA added. All measurements were performed twice.

For each combination of extract type and pH value, data were fitted to a log–logistic equation:  $f(x) = 0 + d / \{1 + \exp(b(\log(x) - \log(e)))\}$ . In the equation, f(x) represents the amount of BSA precipitated in µg, and x the addition of extract in mg. Parameter d equals the maximum precipitation (plateau), and e equals the amount of extract when 50% of d was reached (ED50).

#### **Results and discussion**

Table 1 shows the content of CT and TP for each extract. Especially for quebracho extract, the literature suggested a higher CT content, but only in very few studies was the content actually measured. Bueno *et al.* (2008) found similar CT content (quebracho) to this analysis.

Table 1. Tannin extracts and their content of condensed tannins (CT) and total phenols (TP) in g kg<sup>-1</sup>

	Quebracho	Mimosa	Tara	Gambier
CT	138	253	5	49
TP	ca. 1000	ca. 1000	967	772

Tara plateaus of the precipitation curves partly exceeded the maximum of BSA available in the tube (60 µg). Flocculation was observed in the tara cuvettes after some time, so the measurements may have been affected. Tara plateaus were not statistically different from mimosa plateaus, except at pH 3 and 6 (P < 0.05). Mimosa curves reached maximum precipitation of BSA at all pH conditions and were statistically higher than quebracho plateaus (P < 0.001), except at pH 7. Gambier, on the other hand, always had a smaller slope than the other extracts and often did not even reach a plateau. This may be due to the relatively low CT content. Tara, however, which is mainly a source of hydrolysable tannins and hardly any CT, showed high precipitation activity. Therefore, the data suggest that hydrolysable tannins as well as TP of tara contributed to its precipitation ability.

As apparent in Figure 1 (example precipitation curves are presented for pH 7 and 2), mimosa, tara and gambier plateaus hardly showed any influence of the pH conditions in the analysis. Therefore, it has to be assumed that only quebracho will form pH-dependent complexes with the feed protein and will release the protein again in the abomasum.

Although ED50 can be a parameter of efficiency, it is only comparable among the different extracts in the case of identical plateau levels. ED50 did not show a linear trend. For quebracho it tended to increase with increasing pH value, which is understandable with plateaus becoming higher as well. For mimosa and tara, with mostly identical plateau levels, ED50 was lowest at pH 4 (P < 0.01 for mimosa, P < 0.0001 for tara). Therefore, the BSA precipitation of mimosa and tara seemed to be most efficient at pH 4. However, at pH 7 (P < 0.0001) and pH 2 (P < 0.05), ED50 was lower for mimosa compared with tara, indicating a lower amount of mimosa necessary to precipitate the same amount of BSA. At pH 7, quebracho reached ED50 with smaller amounts of extract than tara, but with higher amounts than mimosa (P < 0.0001). Gambier did not show any influence of pH value on the ED50 (P > 0.05).



Figure 1. Precipitation of bovine serum albumin (BSA) in  $\mu$ g by extracts of quebracho (o), mimosa ( $\Delta$ ), tara (+), and gambier (x) in mg dry matter; examples for pH 7 and pH 2

#### Conclusions

The results indicate that the source of CT in combination with the pH conditions influenced their ability to form complexes with BSA. Other phenols may also have influenced this process. Hence, various tannin extracts are likely to react differently to conditions in the rumen and abomasum. Among the extracts analysed, only quebracho seemed to release the protein at low pH values and this may be most suitable tannin for increasing nitrogen-use efficiency in ruminants. Therefore, quebracho was chosen for a feeding trial with rumen-fistulated heifers during summer 2011.

#### References

Barry T.N. and McNabb W.C. (1999) The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *British Journal of Nutrition* 81, 263–272.

Bueno I.C.S., Vitti D.M.S.S., Louvandini H. and Abdalla A.L. (2008) A new approach for *in vitro* bioassay to measure tannin biological effects based on a gas production technique. *Animal Feed Science and Technology* 141, 151–170.

Cassida K.A., Griffin T.S., Rodriguez J., Patching S.C., Hestermann O.B. and Rust S.R. (2000) Protein degradability and forage quality in maturing alfalfa, red clover and birdsfoot trefoil. *Crop Science* 40, 209–215. Jones W.T. and Mangan J.L. (1977) Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia Scop.*) with Fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* 28, 126–136.

Osborne N.J.T. and McNeill D.M. (2001) Characterisation of *Leucaena* condensed tannins by size and protein precipitation capacity. *Journal of the Science of Food and Agriculture* 81, 1113–1119.

Singleton J. and Rossi P. (1965) Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16, 144–158.

Terrill T.H., Rowan A.M., Douglas G.B. and Barry T.N. (1992) Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of the Science of Food and Agriculture* 58, 321–329.

# Forage production and unique quality characteristics of autumn-grown oat in northern environments

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# Abstract

Oat (*Avena sativa* L.) grown for forage in the northern USA is usually sown in spring and harvested in early summer, with rapid decline in quality after boot stage. Our goals were to determine if there are differences in forage yield and quality between spring-sown summer-harvested and summer-sown autumn-harvested oat, if response to season is similar among cultivars, and optimum sowing date for autumn forage production. Oat sown in early August and harvested 77 days later produced 6.7 Mg ha<sup>-1</sup> dry matter, only 13% less than oat sown in mid-April and harvested 77 days later. Neutral detergent fibre (NDF) was 13% lower and NDF digestibility 38% greater in autumn vs. summer oat forage. Crude protein concentration was 33% greater and water-soluble carbohydrates 150% greater in late-season compared to early-season oat forage. Autumn forage yield was not different among oat cultivars, but large forage quality differences were observed. Delaying summer planting from 2 August to 16 August resulted in a 12% yield reduction and delaying until 30 August resulted in 31 to 67% yield reduction. Oat sown in early August produces high yields of high quality forage, providing an opportunity for double cropping with winter wheat or other early harvested crops.

Keywords: Avena sativa, forage quality, double cropping

# Introduction

In the northern USA oat for forage is sown in spring, usually as a companion crop for perennial forage legume establishment, and harvested in early summer at boot stage maturity for silage. Forage yield from spring-sown oat can range from 5 to 8 Mg ha<sup>-1</sup>, depending on maturity, but neutral detergent fibre (NDF) concentration is typically greater than 500 g kg<sup>-1</sup> and digestibility usually less than 600 g kg<sup>-1</sup> DM. Controlled environment research reported by Smith (1974) demonstrated that a temperature gradient from warm to cold decreases stem proportion and delays maturity. Furthermore, forage digestibility and water-soluble carbohydrate (WSC) concentrations increase when oat is grown under cool temperatures (Smith, 1975). It has been shown that oat accumulates greater amounts of fructans when grown under low temperature, and this response is different among oat cultivars (Livingston and Premakumar, 2002). Preliminary research reported by Maloney et al. (1999) suggests that forage quality of cereal crops could be improved by production under cooler conditions. There is circumstantial evidence to suggest that oat forage production in autumn could be substantially different from spring. Our objectives were to determine whether there are differences in forage yield and nutritive value of oat grown in spring vs. oat grown in autumn, and to determine the effect of August sowing date on forage oat performance.

# Materials and methods

The first experiment was conducted on silt loam soils at University of Wisconsin Agricultural Research Stations near Lancaster and Arlington, WI. Ammonium nitrate fertilizer was applied at a rate of 70 kg ha<sup>-1</sup> before sowing Jim, Gem, and ForagePlus (early, medium and late

maturing) oat cultivars into 3.0 by 6.0 m plots at a rate of 134 kg ha<sup>-1</sup>. Oat was sown on 14 April and 7 August at Arlington and 15 April and 9 August at Lancaster in a split-block design with four replications. Maturity rating (Zadocks' Scale) was made and forage harvested 77 days after sowing and subsamples dried to determine dry matter yield. These were ground and analysed by standard laboratory methods for NDF, neutral detergent fibre digestibility (NDFd), invitro true digestibility (IVTD), crude protein (CP), and water-soluble carbohydrates (WSC). A second experiment was conducted over 2 years at Arlington to determine yield response to August sowing date with similar agronomic practices previously described. Badger and Kame (early), Esker and Vista (medium) and ForagePlus (late) oat cultivars were sown on 2, 16, and 30 August and harvested 20 October 2010 and 24 October 2011.

#### **Results and discussion**

Oat maturity 77 days after sowing differed between seasons and among cultivars (Table 1). In early summer, oat was much more mature than in autumn. Maturity differences among cultivars were much greater in autumn than in early summer, with ForagePlus the least mature and Jim the most mature in early summer (Z71 vs. Z77) and autumn (Z37 vs. Z55). Oat maturity was delayed in autumn, more for cultivars categorized as late maturing than early maturing. Yield differences among cultivars were not detected in early summer or autumn (Table 1), but early summer yields were 1.0 Mg ha<sup>-1</sup> greater than in autumn 77 days after sowing. Greater yield in early summer is associated with more advanced maturity and greater stem production. Precipitation was near normal at both locations and soil moisture was yield limiting in either spring or autumn.

Forage nutritive value differed between seasons and among cultivars (Table 1). Concentrations of NDF were lower in autumn oat than summer oat (521 vs. 596 g kg<sup>-1</sup>) and always lowest in the later maturing ForagePlus. Water soluble carbohydrate concentrations were more than two times greater in autumn than summer, a natural response to cold adaptation even for some annual crops. ForagePlus, with less demand for energy for stem elongation, had the greatest autumn WSC concentration among the oat cultivars. Oat CP concentration was greater in autumn than summer forage and this was associated with less mature and leafier plants in the autumn. In-vitro true digestibility was greater in autumn than summer-harvested oat (796 vs. 671 g kg<sup>-1</sup>) and this is associated with less NDF and more WSC and CP in autumn than summer. ForagePlus had greatest digestibility among the oat cultivars in summer and autumn. Neutral detergent fibre digestibility is currently recognized as an important factor regulating rate of passage and energy availability to ruminants. Cooler temperatures and less mature forage in autumn contributed to much greater NDFd (612 vs. 445 g kg<sup>-1</sup>) compared to summer oat forage. ForagePlus had greater NDFd in both seasons, likely a result of it being less mature and leafier than the other cultivars at all harvests. Acceptable yields of high quality forage can be obtained from summer-sown, autumn-harvested oat, but the amount of time between sowing and harvest (likely at the killing frost) needs clarification.

In the second experiment, rainfall was above normal and oat emergence and oat growth after three August sowing dates was rapid in 2010. Mean yield ranged from 5.7 to 1.9 Mg ha<sup>-1</sup> over the three sowing dates, with only a 0.8 Mg ha<sup>-1</sup> drop in yield associated with delaying sowing from 1 August to 16 August (Table 2). Yield tended to be greatest with Esker and Vista, two intermediate-maturing cultivars. An extended period of below normal precipitation from early July until late August caused delayed and uneven emergence with 2 August and 16 August sowing dates in 2011. This resulted in a shorter period for growth, delayed maturity and forage yield less than half that achieved in 2010. Although the extremely dry July-August period was unusual for Wisconsin, the results demonstrate that

precipitation patterns will have a large effect on the success of obtaining economical forage yield from late season oat.

Cultivar	Matanital	DM Yield	NDF	WSC	СР	IVTD	NDFd				
	Maturity	Mg ha <sup>-1</sup>		g kg-	<sup>1</sup> DM		g kg <sup>-1</sup> NDF				
		April sow	April sowing – early summer harvest								
Jim	77a <sup>2</sup>	7.9a	573b	71c	120f	641f	373e				
Gem	77a	7.7a	595b	67cd	134e	657e	424d				
ForagePlus	71b	7.6a	619a	58d	150d	713d	536c				
August sowing – autumn harvest											
Jim	55c	6.7b	554bc	154b	161c	755c	558bc				
Gem	44d	6.8b	529c	160b	176b	776b	577b				
ForagePlus	37e	6.7b	478d	173a	205a	856a	701a				

Table 1. Forage dry matter yield and nutritive value of three oat cultivars harvested 77 days after sowing in April vs. August at two locations in Wisconsin

<sup>1</sup>Maturitiy rating is with Zadocks' scale.

<sup>2</sup>Means within a column followed by different letters are significantly different at P = 0.05.

Table 2. Effect	of August s	owing date	on dry m	atter yield a	nd maturity o	f oat forage ha	rvested in October
	<u> </u>	<u> </u>	~	2		0	

Cultivor	20	10 Sowing da	ite	2011 Sowing date				
Cultivar	2 Aug.	16 Aug.	30 Aug.	2 Aug.	16 Aug.	30 Aug.		
	Mg ha <sup>-1</sup> DM (maturity)							
Badger	5.4b <sup>2</sup> (75a <sup>3</sup> )	5.2a (59a)	2.4a (37a)	2.5b (57a)	2.6a (50a)	1.8 (39a)		
Kame	5.3b (75a)	4.6b (53a)	1.9bc (34b)	2.4b (55b)	2.2b (39c)	1.7 (37b)		
Esker	5.9a (73a)	5.0ab (41b)	1.6c (34b)	3.2a (55b)	2.4ab (41b)	1.8 (37b)		
Vista	6.2a (57b)	5.1ab (37c)	2.1b (34b)	2.6b (45d)	2.4ab (39c)	1.9 (37b)		
ForagePlus	5.4b (41c)	4.4bc (37c)	1.5c (34b)	2.1b (50c)	2.1b (37c)	1.7 (37b)		
Mean	5.7	4.9	1.9	2.6	2.3	1.8		

<sup>1</sup>Harvest dates were 20 October 2010 and 24 October 2011.

<sup>2</sup>Yield and maturity means within a column followed by different letters are significantly different at P = 0.05.

<sup>3</sup>Maturity rating is with Zadocks' scale.

#### Conclusions

Our results show that oat forage produced in autumn, with cooler temperature and shorter days, has substantially greater nutritive value than oat produced in early summer. Neutral detergent fibre concentrations are lower and NDFd is remarkably greater in autumn compared to early season oat forage. In addition, high WSC concentrations in autumn-harvested oat forage should aid in its fermentation when preserved as silage. Yields tend to be greater with intermediate-maturity cultivars, but nutritive value is greatest in the very late, leafy, ForagePlus. In southern Wisconsin, sowing oat for autumn forage should occur in early to mid-August, allowing a minimum of 65 to 70 days before a killing frost, to optimize yield and quality.

#### References

Maloney T.S., Oplinger E.S. and Albrecht K.A. (1999) Small grains for fall and spring forage. *Journal of Production Agriculture* 12, 488–494.

Livingston D.P. and Premakumar R. (2002) Apoplastic carbohydrates do not account for differences in freezing tolerance of two winter-oat cultivars that have been second phase cold-hardened. *Cereal Research Communications* 30, 375–381.

Smith D. (1974) Influence of temperature on growth of Froker oats for forage. I. Dry matter yields and growth rates. *Canadian Journal of Plant Science* 54, 725–730.

Smith D. (1975) Influence of temperature on growth of Froker oats for forage. II. Concentrations and yields of chemical constituents. *Canadian Journal of Plant Science* 55, 897–901.

# Variability of herbage quality of meadow fescue populations and cultivars

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# Abstract

Meadow fescue (*Festuca pratensis* Huds.) is a perennial grass species that has high yield potential and biomass quality. The collection investigated included five breeding populations and six cultivars. Breeding populations have passed two cycles of selection. In three cuts, dry matter (DM) chemical composition (crude protein (CP), crude fibre, crude fat and ash content) and CP protein yield were analysed. Within-population variability was highest for crude fat content and lowest for crude ash content. Broad sense heritability ( $h_b^2$ ) was high, (over 89%), for all investigated traits. Genetic distance of meadow fescue genotypes studied was represented by cluster analysis. Varieties OP-4, OP-5 and Jabeljska showed high CP content, but due to the lowest DM yield they have shown low CP yield in total. Nevertheless, these genotypes can be a source of genes in breeding for improved quality. Data obtained in this study suggest that some genotypes are potentially valuable for further breeding, and genetically most distanced genotypes will be inter-crossed to produce superior progeny.

Keywords: meadow fescue, DM chemical composition, populations, cultivars, variability

# Introduction

Meadow fescue (*Festuca pratensis* Huds.) is a species of increasing importance, particularly in the northern hemisphere where it can dominate in meadows and pastures (Casler i Santen, 2000). It has been used usually for intensive production of hay meadows. Meadow fescue is also very tolerant of grazing, and therefore pastures where it dominates are long lasting. It can be grown as a pure crop, but more often in mixtures with other grasses and legumes. Meadow fescue is a highly productive forage grass, with high nutritive value (Kölliker, 1998). The herbage quality changes depending on the stage of maturity, but because it flowers late (second half of May), high quality of herbage is retained for a long time. This is a very important characteristic for meadow fescue to be a component in grass-legumes mixtures. In terms of quality and digestibility, meadow fescue can be compared to species of the genus *Lolium* (Kölliker, 1998). The primary aim in this study was to determine the variability and heritability of dry matter (DM) chemical composition traits and to find potential genotypes for further breeding.

# Materials and methods

A collection which consisted of five breeding populations and six cultivars of meadow fescue was investigated during two years (2006 and 2007). The breeding populations passed two cycles of selection. Four originated from the local populations, collected mainly in Eastern Serbia (OP-1, OP-2, OP-3 and OP-4), and the population OP-5 originated from Czech Republic. Cultivars included in this study were: Kruševački 21 (K-21) (Serbia), Jabeljska (Slovenia), Premil, Pradel, Preval (Switzerland) and Rožnovska (Czech Republic). The study was conducted in an experimental field of the Institute for Forage Crops, on a degraded alluvium soil type. The trial was set up in a spaced-plant nursery with plant-to-plant distances of  $60 \times 60$  cm, in a randomized block design with 30 plants per genotype in three replications. Sowing in containers was performed in early spring in the greenhouse and

planting in the experimental field occurred in April 2005. Chemical composition of the DM (crude protein (CP), crude fibre (CF), crude fat and ash) was determined by the Weende system, in three cuts and the results were presented as two-years average values (2006 and 2007). First cut was done after heading (third decade of May), second in first decade of July, and third in first decade of October. Crude protein yield was calculated based on two years average DM yield and average crude protein content. All data were analysed by ANOVA. Differences between genotypes were tested by the LSD test. Cluster analysis was made by the Ward method, using the Euclidian distances (Statistica 5.0, Stat Soft. Inc.). Broad sense heritability  $(h_b^2)$  was calculated according to the formula:

$$h_b^2 = \frac{\delta_g^2}{\delta_f^2} \cdot 100 \ (\delta_g^2 - \text{genetic variance}; \ \delta_f^2 - \text{phenotypic variance}).$$

#### **Results and discussion**

The most important indicator of the DM quality is content of crude protein, which mainly depends on the growth stage at the time of cutting. In this study, the genotypes with highest crude protein content and lowest crude fibre content were OP-4, OP-5 and the cultivar Jabeljska, (Table 1), which gives them characteristics to be used as sources for improved DM quality breeding. Variability within genotypes studied (*CV*%) for all investigated traits was not high, but differences between some genotypes (LSD) were statistically highly significant (Table 1). Broad sense heritability ( $h_b^2$ ) was high for all traits. The lowest heritability was determined for CP content in the first cut (89.85%). For other traits, values of broad sense heritability were substantially higher, which indicates that an improvement in these traits could be possible.

Parameters	Cri	ide prot	ein	C	rude fib	re	(	Crude fat Ash					
Constructs		Cuts			Cuts			Cuts			Cuts		
Genotypes	Ι	II	III	Ι	II	III	Ι	II	III	Ι	II	III	
OP-1	98.8	131.4	124.7	361.0	233.7	212.0	23.7	46.3	39.4	82.8	136.6	120.5	
OP-2	78.1	100.9	112.3	363.6	321.3	268.9	14.8	35.4	23.0	91.4	148.9	118.3	
OP-3	88.5	118.6	131.2	337.2	318.5	258.9	25.8	35.5	26.6	82.8	142.0	129.2	
OP-4	110.6	147.9	175.2	317.7	284.2	236.4	24.2	32.7	39.2	98.0	133.0	130.6	
OP-5	100.0	148.8	161.4	315.6	265.5	180.5	21.0	45.9	43.8	76.1	132.4	146.6	
K-21	105.5	103.5	121.1	380.8	314.4	353.0	18.0	31.9	25.6	85.5	132.5	122.1	
Jabeljska	107.7	151.2	136.6	316.4	300.8	259.5	24.6	44.7	32.3	87.7	137.0	114.6	
Premil	83.5	139.8	134.6	366.1	255.1	200.7	12.4	44.1	27.1	78.6	145.0	111.2	
Pradel	90.1	120.3	123.8	359.1	357.1	186.3	15.5	40.4	29.8	86.5	121.6	112.8	
Preval	103.5	133.6	130.3	345.7	271.0	239.0	23.2	33.7	31.8	85.0	115.3	105.0	
Rožnovska	84.3	129.4	134.5	368.0	275.2	257.6	16.8	29.8	30.0	81.3	126.1	110.7	
Average	95.5	129.2	135.0	348.3	290.6	241.6	20.0	38.2	31.6	85.1	133.6	120.1	
CV (%)	11.57	13.1	13.43	6.68	12.21	20.05	7.08	16.2	20.66	7.08	7.45	9.78	
LSD 0.05	10.6	24.5	20.1	31.2	28.4	26.3	8.6	13.1	10.8	17.6	27.3	20.8	
LSD 0.01	12.2	30.2	31.3	46.7	37.6	36.1	10.9	16.2	13.6	19.5	35.4	28.3	
$h_{h}^{2}(\%)$	89.85	99.73	99.43	99.2	99.33	99.81	97.89	99.6	99.78	98.9	97.99	98.47	

Table 1. Dry matter chemical composition (g kg<sup>-1</sup>) of meadow fescue genotypes

Crude protein yield is an important indicator of agronomic value of genotypes, because it depends on DM yield and quality simultaneously. Since the DM yield of the first cut represents the major part of the annual yield of dry matter, CP yield in the first cut is the greatest part of the total CP yield, although the content of CP in the regrowth is higher than in the first cut. The highest CP yield was recorded in cultivars Preval, K-21 and Pradel (Table 2). In the study of Niemeläinen *et al.* (2001), average annual CP yield of meadow fescue cultivars ranged from 1191 to 1331 kg ha<sup>-1</sup> which is in agreement with our results.

Table 2. Crude protein yield (kg ha-1) of meadow fescue genotypes

Gen.	OP-1	OP-2	OP-3	OP-4	OP-5	K-21	Jab.	Premil	Pradel	Preval	Rož.	Average
I cut	628.8	652.2	401.1	530.3	636.2	855.7	584.1	596.6	755.9	822.7	673.3	648.8
II cut	140.8	152.7	153.9	133.5	161.0	155.5	130.8	169.6	200.8	218.8	179.9	163.4
III cut	83.3	108.5	102.1	81.25	114.7	118.9	62.7	93.9	118.0	109.0	119.1	101.04
Total	852.9	913.4	657.1	745.1	911.9	1130.1	777.6	860.1	1074.1	1150.5	972.3	913.24

Cluster analysis was performed in order to determine the genetically most distanced genotypes for further crosses and for obtaining superior progenies. Genotypes were clustered into two main groups (Figure 1) and the first group was composed of two subgroups. In the first subgroup are genotypes with medium content of CP and high of crude fibre (1a). The second subgroup consisted of genotypes that are characterized by low content of CP and highest crude fibre content (1b). The second group consists of genotypes that have the highest CP and low crude fibre content (2)



Figure 1. Cluster diagram of meadow fescue genotypes based on DM chemical composition

# Conclusions

Variability within the genotypes examined for all studied traits was not high. On the other hand, heritability was very high. Statistically significant differences between the studied genotypes of meadow fescue were found by ANOVA for all investigated traits. OP-4, OP-5, Jabeljska and Preval showed the high crude protein content and they can be a source of genes in breeding for improved quality. Values of analysed traits suggest that some genotypes are potentially valuable for further breeding, and cluster analysis indicated the genetically most distanced genotypes which will be inter-crossed to produce superior progeny.

# Acknowledgment

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# References

Casler M.D. and Santen E.V. (2000) Patterns of variation in a collection of meadow fescue accessions. *Crop Science* 40, 248–255.

Kölliker R. (1998) Genetic variability in *Festuca pratensis* Huds.: Effect of management on natural populations and comparison of cultivars to other species. A disertation submitted to the Swiss Federal Institute of Technology, Zurich, 1–82.

Niemeläinen O., Jauhiainen L. and Miettinen E. (2001) Yield profile of tall fescue (*Festuca arundina-cea*) in comparison with meadow fescue (*Festuca pratensis*) in Finland. *Grass and Forage Science* 56, 249–258.

# Critical P concentration of timothy and multi-species swards

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#### Abstract

Plant-based diagnostic methods require the definition of critical nutrient concentrations. The dilution of P and N in increasing shoot biomass has led to the development of a model of critical P concentration in which critical P concentrations during the growing season are predicted as a function of N concentrations. Our objective was to validate an existing model of critical P concentration ( $P_c = 1.07+0.063N$ ) established for timothy (*Phleum pratense* L.) in eastern Canada. Experiments with varying rates of P fertilization were conducted in 2010 on soils with a low available P content at sites with timothy in Canada (Normandin and Quebec) and Finland (Maaninka), and at sites with multi-species swards in Switzerland (Changins) and France (Toulouse). Dry matter (DM) yield, and N and P concentrations were measured on four dates with one-week intervals from the vegetative to late heading stages of development. Increasing P fertilization had a positive effect on DM yield at only one (Toulouse) of the five locations. Non-limiting P conditions were therefore reached with no P applied at Normandin, Québec, Maaninka, and Changins, and with 50 kg P ha<sup>-1</sup> at Toulouse. Concentrations of N and P under these non-limiting P conditions confirmed the validity of the model of critical P concentration for timothy but not for multi-species swards.

Keywords: phosphorus, grasses, deficiency, model

# Introduction

Plant-based methods of identifying P and N deficiencies depend on the definition of optimal or critical concentrations, that is, the minimum concentration of a given nutrient required to achieve maximum shoot growth and yield. Both P and N concentrations in crops decrease with increasing shoot biomass and advancing maturity. Hence, the definition of critical P and N concentrations should take this decrease into account. The concept of P dilution and its use for the development of a model of critical P concentration were first proposed for perennial grasses in Europe (Salette and Huché, 1991; Duru *et al.*, 1997). They were also investigated for timothy in eastern Canada (Bélanger and Richards, 1999; Bélanger and Ziadi, 2008). This model of critical P concentrations during the growing season are then predicted as a function of N concentrations. A model of critical P concentration ( $P_c$ ) was developed for timothy in eastern Canada under conditions where P was assumed not limiting for growth ( $P_c = 1.07+0.063N$ ; Bélanger and Ziadi, 2008). The use of this model of critical P concentration in situations of P deficiency has not yet been assessed. Our objective was

to validate an existing model describing the critical P concentration in shoot biomass during the growing season.

#### Materials and methods

Experiments were conducted at five locations on soils with low available soil P content according to local soil testing. Experiments with timothy in first production year were conducted at three of the locations (Normandin and Quebec, Canada; Maaninka, Finland). At the other two locations (Changins, Switzlerland; Toulouse, France), long-term P fertilization studies with multi-species grasslands were used. In the timothy-based experiments, four P rates (0, 10, 20, and 40 kg P ha<sup>-1</sup>) were applied in spring whereas we used three P rates (0, 8, and 17 kg P ha<sup>-1</sup>) in Changins and two P rates (0 and 50 kg P ha<sup>-1</sup>) in Toulouse. Nitrogen and potassium were applied to ensure non-limiting conditions. A split-plot design was used with P rates as main plots and sampling dates as subplots with four replications at four of the sites and three replications in Toulouse. Sampling during the spring regrowth was done on four sampling dates, one week apart from vegetative to late heading stages of development. At each sampling date, dry matter (DM) yield was measured by cutting at a 5-cm height an area of at least 1 m<sup>2</sup>. A fresh sample of around 300–500 g was taken in each plot, dried at 55°C for 3 d, and ground to 1-mm. Plant samples were analysed for P and N concentrations.

#### **Results and discussion**

Increasing P fertilization did not significantly (P > 0.05) affect DM yield on all sampling dates of the spring regrowth in the three timothy-based experiments (Figure 1). In Toulouse, P fertilization significantly ( $P \le 0.05$ ) increased DM yield on all four sampling dates whereas in Changins, P fertilization significantly ( $P \le 0.05$ ) increased DM yield on the first two sampling dates but decreased it on the last two sampling dates. Although forage grass DM yield does not always respond to P fertilization (Grant and MacLean, 1966; Bélanger and Ziadi, 2008; Valkama *et al.*, 2009), the lack of a DM yield response to P fertilization at most locations was surprising. Locations were chosen because of their expected positive response to P fertilization puts into question the validity of the soil P test and its interpretation, and confirms the need for improved methods for predicting P requirements of forage grasses. To validate the model of critical P concentration, we considered that non-limiting P conditions were obtained when no P was applied at the three timothy-based experiments and



Figure 1. Effect of P fertilization on DM yield of timothy (Quebec, Normandin, and Maaninka) and multi-species swards (Changins and Toulouse) on the third sampling date of the spring regrowth

in Changins, and with 50 kg P ha<sup>-1</sup> in Toulouse. Under these non-limiting P conditions, the relationship between P and N concentration was similar to that previously reported by Bélanger and Ziadi (2008) at the three locations with timothy-based experiments (Figure 2). In Changins, however, P concentrations for a given N concentration were much lower than at the other sites while much higher values were observed at Toulouse.



Figure 2. Relationship between P and N concentrations of timothy (Quebec, Normandin, and Maaninka) and multi-species swards (Changins and Toulouse) grown under non-limiting P conditions and the model of critical P concentration published by Bélanger and Ziadi (2008)

#### Conclusions

The model of critical P concentration proposed by Bélanger and Ziadi (2008) was therefore confirmed for timothy but not for multi-species swards. The model of critical P concentration provides an essential tool for calculating an index of P nutrition as the ratio of P concentration to the critical P concentration.

#### References

Bélanger G. and Richards J.E. (1999) Relationship between P and N concentrations in timothy. *Canadian Journal of Plant Science* 79, 65–70.

Bélanger G. and Ziadi N. (2008) Phosphorus and nitrogen relationships during spring growth of an aging timothy sward. *Agronomy Journal* 100, 1757–1762.

Duru M. and Ducrocq H. (1997) A nitrogen and phosphorus herbage nutrient index as a tool for assessing the effect of N and P supply on the dry matter yield of permanent pastures. *Nutrient Cycling in Agroecosystems* 47, 59–69.

Grant E.A. and MacLean A.A. (1966) Effect of nitrogen, phosphorus, and potassium on yield, persistence, and nutrient content of timothy. *Canadian Journal of Plant Science* 46, 577–582.

Salette J. and Huché L. (1991) Diagnosis of the mineral nutrition status of a pasture through herbage analysis: principles, implementation, examples. (In French, with English abstract.) *Fourrages* 125, 3–18. Valkama E., Uusitalo R., Ylivainio K., Virkajarvi P. and Turtola E. (2009) Phosphorus fertilization: A meta-analysis of 80 years of research in Finland. *Agriculture, Ecosystems and Environment* 130, 75–85.
# Characteristics of red clover (*Trifolium pratense* L.) populations collected in West- and North-West Estonia

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## Abstract

During the expeditions organised in West- and North-West Estonia in 2002, seeds of natural red clover populations were gathered. Two field trials of these populations were established at the Jõgeva Plant Breeding Institute: one (2003) for the assessment of development rate and seed yield, and the other (2006) for the evaluation of dry matter yield and its quality in a three-cut utilization regime. Data were collected from both trials during two years. Of yield quality indicators, crude protein content was determined from a general sample. Based on further sampling, the percentage of alien species in the harvested biomass was determined as well as the ratio of leaves and stalks in red clover biomass, and crude protein content in biomass fractions. The early diploid red clover cultivar Jõgeva 433 was used in the trials as a control variety. The aim of the research was to estimate the breeding value of red clover populations in Estonia to find out the potential for utilization. Altogether 22 natural red clover populations were studied.

Keywords: natural populations, seed and dry matter yield, herbage quality

## Introduction

In Estonia, grassland husbandry research centred on using natural populations of red clover in the first half of the 20<sup>th</sup> century, when fodder for herbivores was mostly obtained from semi-natural grasslands. In the past decades, natural populations of red clover have become the centre of attention in the context of preservation of genetic resources and the production of forage crop seed mixtures aiming at the preservation of the natural environment. The present research was initiated under the programme of collection and preservation of genetic resources of plant species, aiming to evaluate and describe the morphological, biological and agronomical properties of the collected material. The objective was to identify in field trials the properties of natural red clover populations collected during the expeditions in West- and North-West Estonia and to compare them with cultivars. Up to recent time, several authors (Novosjolova *et al.*, 1978; Cope and Taylor, 1985; Boller *et al.*, 2003; Mrfat-Vukelić *et al.*, 2003; Nedelnik *et al.*, 2003; Hermann *et al.*, 2005) have paid much attention to natural populations and local varieties of red clover to find useful traits such as disease resistance, winter hardiness, longevity, higher seed yield, higher percentage of leaves in the herbage, lower fibre content in leaves and stalks, etc.

## Materials and methods

In the expeditions organised in 2002, red clover seeds were harvested in natural habitats and old abandoned cultivated meadows and pastures. This enabled us to establish two plot trials, one for the determination of seed yield (one replication, established in 2003) and the other one for dry matter (DM) yield assessment (4 replications, randomized block design, 2006). Both trials were established on black fallow in the first half of July, using narrow-row seeding at a rate of 12 kg of scarified seed per hectare; they were exploited for two years. The trials were conducted on a calcaric cambisol (Ko), pH <sub>KCI</sub> was 6.2, C organic

matter content 21 g kg<sup>-1</sup>, total nitrogen content (N) 1.3 g kg<sup>-1</sup>, P 230 mg kg<sup>-1</sup>, K 229 mg kg<sup>-1</sup>, Ca 1,550 mg kg<sup>-1</sup> and Mg 77 mg kg<sup>-1</sup>. Prior to the trial establishment, mineral fertilizers were applied at a rate of 19 kg ha<sup>-1</sup> of P and 67 kg ha<sup>-1</sup> of K. The trial established for the determination of herbage yield and quality was managed under a three-cut regime, and cutting time was determined by the beginning of flowering of the control cultivar 'Jõgeva 433'. In the laboratory, crude protein in the DM was determined (Kjeldahl N × 6.25) as well as the leaves-stalks ratio in the harvested biomass and crude protein content in biomass fractions. The studied red clover populations are named in this paper according to the place of collection. Data processing was carried out by AGROBASE, using analysis of variance.

#### **Results and discussion**

The growth and development of red clover populations gathered from semi-natural grasslands of West- and North-West Estonia was similar to that of early red clover cultivars (onset of flowering in spring growth on 15 June). Among the studied accessions there was a population collected in the vicinity of Hullo village on the island of Vormsi, which started flowering 5 days earlier than the control variety Jõgeva 433 and which could be useful for breeding even earlier red clover varieties than those currently in production. However, modest seed-yields and high number of white-flowered plants in this population are impediments for further breeding.

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		Latitude	Longitude	Dry matter	yield	Crude protei	n yield	Seed	yield
				Mg ha <sup>-1</sup>	%	kg ha <sup>-1</sup>	%	kg ha <sup>-1</sup>	1000 s g
1	Jõgeva 433			20.44 a	100.0	3,877 a	100.0	173.8	1.802
2	Jõgeva 205			19.23 ab	94.1	3,571 abc	92.1	145.7	1.796
3	Selja	58°32'51"	24°50'47"	19.45 ab	95.2	3,627 ab	93.6	189.1	1.834
4	Juurikaru	58°38'10"	25°06'00"	18.35 dc	89.8	3,533 abc	91.1	140.4	1.718
5	Hanila	58°37'10"	23°37'43"	17.17 cd	84.0	3,436 bcd	88.6	116.3	1.495
6	Ridala	58°54'46"	23°28'02"	16.99 cde	83.1	3,454 bcd	89.1	106.9	1.541
7	Reigi	58°59'00"	22°30'42"	16.51 cdef	80.8	3,192 defg	82.3	104.3	1.642
8	Pürksi	59°01'06"	23°31'10"	16.37 defg	80.1	3,192 defg	82.3	122.0	1.501
9	Jämaja	58°00'47"	22°03'30"	15.89 defgh	77.7	3,124 defg	80.6	109.7	1.604
10	Lihula	58°43'33"	23°55'28"	15.78 defghi	77.2	3,236 cdef	83.5	97.7	1.461
11	Kärdla	58°59'27"	22°46'08''	15.77 defghi	77.2	3,215 cdef	82,9	98.5	1.545
12	Vormsi	58°58'49"	23°15'24"	15.63 defghi	76.5	3,042 efgh	78.5	58.9	1.578
13	Nõmmküla	58°40'09"	23°12'28"	15.61 defghi	76.4	3,163 defg	81.6	122.5	1.547
14	Hämmelepa	58°17'45"	22°46'44"	15.61 defghi	76.4	3,148 defg	81.2	97.7	1.442
15	Sääre	57°54'37"	22°03'17"	15.45 defghi	75.6	2,925 fgh	75.4	71.5	1.400
16	Varbla			15.28 efghij	74.8	2,973defg	76.7	126.2	1.531
17	Käina	58°50'06"	22°47'18"	15.15 efghij	74.1	2,887 ghi	74.5	121.5	1.724
18	Tõlluste	58°24'20"	22°59'50"	15.11 efghij	73.9	3,176 defg	81.9	86.6	1.516
19	Virtsu	58°34'44"	23°33'23"	14.72 fghij	72.0	2,923 fgh	75.4	101.2	1.468
20	Salme	58°10'44"	22°06'35"	14.35 hij	70.2	2,787 hij	71.9	79.5	1.434
21	Karujärve	58°22'44"	22°14'05"	13.38 jkl	65.5	2,514 ijk	64.8	101.4	1.367
22	Osmussaar I	59°16'51"	23°24'18"	12.26 kl	60.0	2,426 jk	62.6	138.5	1.561
23	Metsküla	58°43'31"	23°36'55"	12.20 kl	59.7	2,513 ijk	64.8	116.0	1.453
24	Osmussaar II	59°18'09"	23°22'22"	11.531	56.4	2,364 k	61.0	70.7	1.383
	LSD 95%			1.82		363			

Table 1. Herbage and crude protein yields of natural red clover populations as the total of two harvest years (2007, 2008) and seed yield as a mean of two harvest years (2004, 2005)

Mean values not sharing a common letter are significantly different.

The seed-yields of natural red clover populations were inferior to those of cultivars, being 20–66% lower (Table 1). The 1000 seed weight was 0.2–0.4 g lower than that of cultivars. Among the populations included in the trials, the population collected from the shingly central part of Osmussaare I attracted attention by its two-year average seed yield of 138.5 kg ha<sup>-1</sup> (79.7% of the control variety). This accession, with modest DM yield but abundant flowering, could be used for landscaping (road verges, flowering park meadows, flower lawns).

In cultivated grasslands that have remained unused for years, late red clover can be also found (e.g. Selja and Juurikaru), beginning of flowering in spring growth on 7 July. In the late red clover population collected from Selja in Pärnumaa, the average seed yield of two harvest years exceeded the control variety by 8.8%, which indicates the need of further investigations to find out why the individuals survive so long and they have unexpectedly high seed-yielding ability and stability over years.

Under a three-cut regime the natural red clover populations had 16–44% lower DM yield and digestible dry matter yield, and 12–39% lower crude protein yield than the control variety Jõgeva 433. Among the natural populations the one collected from Hanila was by 16.0% inferior to the control variety in DM and by 11.4% in crude protein yield.

In the biomass of all three cuts the proportion of leaves was higher than that of the control variety. Compared to the cultivar Jõgeva 433, there was no significant difference in leaf protein content; however, the stalks of the natural populations in the first cut contained more protein. The population collected along the Varbla road had in the first cut more leaves (+2.4%) and higher protein content, both in leaves (+0.54%) and stalks (+1.04%) compared with control variety Jõgeva 433.

#### Conclusions

For improving the species richness, yielding ability, and forage quality of semi-natural communities through top-seeding, it will be suitable in the future to multiply the late red clover population from Selja and the early one from Hanila. For grasslands to be established for non-forage purposes and for the improvement of ornamental value of semi-natural grass-lands, it will be reasonable to use in the seed mixtures the early red clover population from Osmussaare. Based on the trial results, it can be concluded that among the natural red clover populations, there are forms that are of potential interest to breeders, but the use of them in breeding programmes for fodder production may be difficult due to their tendency to lodge easily and their modest yielding ability.

## References

Boller B., Tanner P., Günter S. and Schubiger F.X. (2003) Description and evaluation of a collection of formerSwiss red clover landraces. *Czech Journal Genetic and Plant Breeding*, Proceedings 25<sup>th</sup> EUCARPIA fodder crops and amenity grasses section meeting 39, 31–37.

Cope W.A. and Taylor N.L. (1985) Breeding and genetics. In: Taylor N.L. (ed.) *Clover science and technology*, Madison, Wisconsin, pp. 383–404.

Hermann D., Boller B., Widmer F. and Kölliker R. (2005) Optimization of bulked AFLP analysis and its application for exploring diversity of natural and cultivated populations of red clover. *Genome* 48(3), 274–286. Mrfat-Vukelić S., Tomić Z., Lazarević D. and Pudlo V. (2003) Grasslands of Serbia as natural genetic resources of forage leguminous plants. *Czech Journal of Genetics and Plant Breeding*. Proceedings 25<sup>th</sup> EUCARPIA fodder crops and amenity grasses section meeting 39, 221–223.

Nedelnik J., Hauptvogel P., Drobna J., Vymyslicky T., Pelikan J. and Hofbauer J. (2003) Potential use of genetic resources fodder crops from Czech and Slovak regions. *Czech Journal of Genetics and Plant Breeding*. Proceedings 25<sup>th</sup> EUCARPIA fodder crops and amenity grasses section meeting 39, 224–227.

Novosjolova et al., 1978; Новосёлова А.С., Константинова А.М., Кулешов Г.Ф., Смурыгин М.А., Ежанова О.Ф. and Михайличенко Б.П. (1978) Селекция и семеноводство многолетних трав. Москва, 303 с.

## Relationships between feed composition characteristics and intake of fresh and ensiled grass-legume mixtures by dairy cows

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## Abstract

An attempt to estimate the correlation between voluntary dry matter intake (VDMI) of legume-grass mixtures given as fresh forages or silages, and different feed compounds, organic matter digestibility and silage quality characteristics, was made on the basis of our own data and literature data. The milk output of cows fed fresh forages or silages as a sole feed in all analysed experiments amounted to 18-22 kg. Intake of dry matter from fresh and preserved grass-legume mixtures was positively correlated with the organic matter digestibility (r = 0.4797 and 0.5777, respectively) and water-soluble carbohydrates content in silages (r = 0.7237) and inversely proportional to the ADF content in fresh fodders (r = -0.6529) and to the acetic acid content in silages (r = -0.6705).

Keywords: voluntary dry matter intake, dairy cows, grass-legume mixtures

## Introduction

The rate of milk production of dairy cows as ruminants depends on both the feed and especially dry matter (DM) intake (Martens, 1994). The particularly important issue is to maximize the DM intake so as to maintain proper functions of the digestive tract and also allow reduced costs of feeding (more forages = less concentrates). The main forages in the diets offered to dairy cows kept on farms that have large areas of permanent grasslands are grasses, legumes and especially high-yielding mixtures of these. Determination of voluntary dry matter intake (VDMI) directly with animals (*in vivo*) makes possible to obtain the most reliable data; however, these procedures are laborious and expensive. Taking the above into consideration, many authors (Wilkins *et al.*, 1971; Rook *et al.*, 1991; Steen *et al.*, 1998; Huhtanen *et al.*, 2002) have tried to develop mathematical models for predicting the DM intake based on simple analysis of chemical composition. The obtained results have differed to a great extent, and the cited authors emphasized the necessity of perpetual improvement of such models by the inclusion of greater number of observations. This paper is an attempt at supplementation of the necessary data. Fresh forages were specially considered because of a lack of data related to those feeds.

## Materials and methods

The correlation between VDMI of fresh or ensiled grass-legume mixtures fed to dairy cows and various nutrients and analyses: NDF, ADF, organic matter digestibility (OMD), and silage quality characteristics (water soluble carbohydrates – WSC, organic acids, pH, ammonium nitrogen, ethanol) was estimated on the basis of the results of our own research and supported by the literature data. The fresh forages used in our own experiments were harvested during 1989–1996 (8 published papers) and the data related to the silages were obtained for the period of 1971–2007 (1 own and 6 foreign papers). The milk output of cows fed fresh forages or silages as a sole feed in all analysed experiments was in the range 18–22 kg. The correlations were statistically analysed using Statistica software.

#### **Results and discussion**

The positive highly significant correlation between VDMI and OMD was found in both fresh forages and silages (Table 1). A similar dependency in fresh grass silages was revealed by Huhtanen *et al.* (2002). The levels of all types of fibre were inversely correlated with VDMI but the correlation was statistically significant ( $P \le 0.05$ ) only in the case of ADF content in fresh forages (Table 1). Expected highly significant correlations were confirmed between VDMI and WSC content of silages (Table 1). There were no significant relations between VDMI and silage quality indices, except for acetic acid where an inverse significant correlation was found.

Correlation coefficients for fresh forages between voluntary dry matter intake and:										
	range of values	r	Р							
Dry matter	116.2–247.9 g kg <sup>-1</sup>	-0.406	0.7542							
Crude fat	30.7–47.9 g kg <sup>-1</sup> DM	0.0763	0.5655							
Crude protein	131.8–210.0 g kg <sup>-1</sup> DM	0.1123	0.1962							
Crude fibre	184.8–317.5g kg <sup>-1</sup> DM	-0.1148	0.1916							
N-free extract	383.0–484.8 g kg <sup>-1</sup> DM	0.0618	0.6419							
Organic matter digestibility	61.3-78.6%	0.4797	< 0.0001							
ADF	242.1–354.6 g kg <sup>-1</sup> DM	-0.6529	0.0407							
Correlations coefficier	ts for silages between volur	ntary dry matter inta	ake and:							
Dry matter	209.4–257.1 g kg <sup>-1</sup>	0.2541	0.2203							
Crude fat	45.6–49.1 g kg <sup>-1</sup> DM	-0.3835	0.6165							
Crude protein	140.5–196.0 g kg <sup>-1</sup> DM	0.1239	0.5552							
Crude fibre	201.2–367.3 g kg <sup>-1</sup> DM	-0.778	0.8220							
N-free extract	304.2–410.3 g kg <sup>-1</sup> DM	0.712	0.2880							
Organic matter digestibility	64.0-77.7%	0.5777	0.0076							
ADF	267.3–363.4 g kg <sup>-1</sup> DM	-0.0859	0.7802							
NDF	375.1–533.3 g kg <sup>-1</sup> DM	-0.0291	0.9005							
Lignin	22.0–57.6 g kg <sup>-1</sup> DM	-0.159	0.7069							
Water soluble carbohydrates	16.5–61.3 g kg <sup>-1</sup> DM	0.7237	0.0007							
pH	3.85–4.38 g kg <sup>-1</sup> DM	-0.1441	0.5120							
Lactic acid	23.5–107.0 g kg <sup>-1</sup> DM	0.0655	0.7557							
Acetic acid	13.1–44.4 g kg <sup>-1</sup> DM	-0.6705	0.0002							
Butyric acid	0.91–5.1 g kg <sup>-1</sup> DM	-0.425	0.0618							
N-NH <sub>3</sub>	4.25–14.4 % N total	0.4325	0.1074							
Ethanol	1.3–6.6 g kg <sup>-1</sup> DM	-0.4576	0.1836							

Table	1.	The	results	of	correlation	anal	vsis
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## Conclusions

The VDMI from eaten fresh and/or ensiled grass-legume mixtures was highly positively correlated with digestibility of organic matter of these forages, and with water-soluble carbohydrates content in silages. Moreover, the VDMI was inversely proportional to the ADF content in fresh forages and to the acetic acid content in silages.

## References

Huhtanena P., Khalili H., Nousiainen J.I., Rinne M., Jaakkola S., Heikkilä T. and Nousiainen J. (2002) Prediction of the relative intake potential of grass silage by dairy cows. *Livestock Production Science* 73, 111–130. Martens D.R. (1994) Regulation of forage intake. In: *Forage Quality, Evaluation and Utilization*. Madison, 450–493. Rook A.J., Gill M., Willink R.D. and Lister S.J. (1991) Prediction of voluntary intake of grass silages by lactating cows offered concentrates at a flat rate. *Animal Production* 52, 407–420.

Steen R.W.J., Gordon F.J., Dawson L.E.R., Park R.S., Mayne C.S., Agnew R.E., Kilpatrick D.J. and Porter M.G. (1998) Factors affecting the intake of grass silage by cattle and prediction of silage intake. *Animal Science* 66, 115–127. Wilkins R.J., Hutchinson K.J., Wilson R.F. and Harris C.E. (1971) The voluntary intake of silage by sheep. I. Interrelationships between silage composition and intake. *Journal of Agricultural Science* 77, 531–537.

## The use of an oxygen barrier stretch film to wrap baled silages in farm conditions

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#### Abstract

Three trials were conducted on commercial farms with the aim of reducing the amount of plastic consumed without reducing the conservation quality of baled silage by using a new oxygen high barrier (HB) film instead of commercial polyethylene (PE) film. Bales of lucerne (DM concentration from 400 to 700 g kg<sup>-1</sup>) were individually wrapped with six layers of conventional PE stretch film or 4 layers of PE-EVOH (Soarnol®) coextruded high oxygen barrier film. The bales were stored outdoors for 330 d. Data confirmed that four layers of high oxygen barrier film could improve hygienic quality of baled silage compared to six layers of commercial PE film, by reducing surface mould development in two out of three trials under farm conditions.

Keywords: oxygen barrier film, baled silage, lucerne, yeast and mould count

#### Introduction

Baled silage is based on a well established procedure that usually consists of wilting forage up to 600 g dry matter (DM) kg<sup>-1</sup>, baling, and then wrapping it with 4 to 6 layers of a stretch polyethylene film. Air-tightness of the bale is decisive to the success and stability of silage kept for extended periods of time (Paillat and Gaillard, 2001). Due to its suitable mechanical characteristics and low costs, polyethylene (PE) has been used for many years for the production of stretch films, but the high oxygen  $(O_{3})$  permeability of PE seems to be one of the main drawbacks of wrapped silage, especially for conservation periods longer than 6 months (Borreani and Tabacco, 2008). The penetration of air into the silage stimulates aerobic bacteria, yeasts and moulds, and causes aerobic deterioration, which results in DM and nutrient losses, the accumulation of pathogens/mycotoxins, and reduced DM intake. Increasing the number of film layers improves air-tightness of the bale coverage and reduces mould growth (Keller et al., 1998). However, this involves prohibitive increases in costs and also environmental concerns due to the disposal of the additional plastic. In confined experiments, Borreani and Tabacco (2010) showed that the use of four layers of a new-generation stretch film, with an enhanced O<sub>2</sub> barrier, reduced DM losses and mould spoilage in comparison to six layers of a commercial PE film. The same authors concluded that new investigations should be undertaken to evaluate the effectiveness of these new barrier films under commercial farm conditions, where bales will be stored outdoors and thereby subjected to different environmental conditions. Hence, this research aimed to study the effects of the type of stretch film (conventional PE and a new coextruded film with an enhanced oxygen barrier properties) on fermentation quality, microbiological counts and mould development on the bale surface of lucerne (Medicago sativa) baled silages under farm conditions.

#### Materials and methods

The experiment was conducted in the North-western Po plain of Italy on 3 commercial farms that usually produce lucerne baled silages. On each farm, one trial (Trial I, II, and III) was conducted to support the objective of reducing the amount of plastic consumed without reducing

the conservation quality of baled silage by using four layers of the new oxygen high barrier film instead of six layers of PE film. On each farm a fourth cut of lucerne was mown, rubber-roll conditioned, spread over the whole field surface and baled within two days at a DM concentration ranging from 400 to 700 g kg<sup>-1</sup>. The forages were baled in 1200 mm-diameter and 1200 mm-long round bales (fixed chamber, no knives in the pickup, net wrapping), transported to the storage area, and individually wrapped with six layers of conventional PE stretch film (co-extruded multi-layer PE film, 500 mm wide  $\times$  25 mm thick, Trioplast Industrier AB, Smålandsstenar, Sweden, 1.5 stretch, light green colour, one year UV resistance certified), or 4 layers of PE – EVOH (Soarnol® special EVOH grade SG611) coextruded high oxygen barrier film (HB, white, UV protected, 500 mm wide  $\times$  25 mm thick, 1.5 stretch). Four bales were wrapped for each treatment in each trial. The bales were stored outdoors on their flat end for 330 d. At opening, the plastic film of each bale was examined carefully for visible holes or damages. On removal of the plastic films, all visible moulds on the bale surface were located and measured, according to Borreani and Tabacco (2010). Four cores were taken from each bale from a depth of 0 to 120 mm (outer layer) and 121 to 540 mm (inner layer) with a core sampler (45 mm diameter) where no mould was visible. Samples were also taken from a depth of 0 to 50 mm from the spoiled bale surface (moulded spot) when present. Silage samples were analysed for DM content, pH, fermentative products, and mould and yeast counts. The data were analysed for their statistical significance by analysis of variance, with their significance reported at a 0.05 probability level using the general linear model of the Statistical Package for Social Science (v 11.5, SPSS Inc., Chicago, Illinois, USA). The mould surface coverage data, expressed as a bale surface percentage, were analysed by the Mann-Whitney non-parametric test of SPSS.

#### **Results and discussion**

At opening, several holes were found in the plastic film in the bales of Trial I, probably due to bird damage (8 and 11.8 holes for PE and HB, respectively). Four layers of HB film significantly reduced the bale surface covered by mould in comparison to six layers of PE film in Trials I and III (Figure 1), whereas mould coverage was comparable in Trial II. The PE film treatment had 20% more of the surface area covered by mould compared to the HB film in Trial I and values lower than 10% in Trials II and III, whereas the HB film reduced the moulded surface below 7% in Trials I and II and any mould spot was present in Trial III. The DM content of silages, fermentation products, pH, yeast and mould counts of inner and



Figure 1. Percentage of bale surface covered by visible moulds in relation to the stretch film (PE, commercial polyethylene, and HB, high barrier film) utilized for wrapping lucerne baled silages after 330 d of conservation, in Trials I to III

outer layers and of the mould spots are reported in Table 1. No significant effect on silage fermentation and microbial quality of the film type was observed in Trials I and II, whereas HB reduced the amount of butyric acid and lowered yeast and mould counts in Trial III. The pH and the mould count were affected by the sampling zone in all the trials, with moulded spot showing the highest values.

Table 1. Fermentative profiles, pH, yeast and mould counts in relation to the bale sampling zon	ıe
(outer layer, inner layer, moulded spot) and the type of stretch film utilized in lucerne baled silag	es
after 330 d of conservation, in Trials I to III	

		HB film				PE film				Effect		
Trial	Item <sup>1</sup>	Outer	Inner	Moulded	Outer	Inner	Moulded	Б	7	E.7	<b>SE</b>	
		layer	layer	spot	layer	layer	Spot	Г	Z	Γ×Ζ	SE	
Ι	DM content	547	552	520	560	542	505	ns	**	ns	10.4	
	pН	5.74	5.66	7.61	5.66	5.67	7.52	ns	***	ns	0.094	
	Lactic acid	7.32	8.14	4.37	8.66	7.74	1.90	ns	ns	ns	0.138	
	Acetic acid	3.95	3.84	2.98	4.54	4.25	3.07	ns	ns	ns	0.493	
	Butyric acid	< 0.01	< 0.01	< 0.01	$<\!0.01$	$<\!0.01$	0.25	_	_	_	—	
	Yeast count	5.44	3.83	6.43	5.65	5.74	6.90	ns	**	ns	0.251	
	Mould count	3.75	3.13	7.18	4.51	3.77	6.44	ns	**	ns	0.329	
II	DM content	727	707	616	682	667	618	ns	ns	ns	11.8	
	pН	5.60	5.53	6.59	5.61	5.45	6.54	ns	***	ns	0.087	
	Lactic acid	0.44	1.81	0.56	0.47	3.73	1.02	ns	*	ns	0.519	
	Acetic acid	3.18	8.88	1.89	4.58	5.04	5.41	ns	ns	ns	0.840	
	Butyric acid	< 0.01	< 0.01	0.14	< 0.01	$<\!0.01$	< 0.01	_	_	_	—	
	Yeast count	2.84	2.45	5.25	2.60	2.37	5.37	ns	**	ns	0.274	
	Mould count	2.56	2.51	5.85	3.34	3.06	5.06	ns	***	ns	0.227	
III	DM content	422	409	_2	400	393	431	ns	ns	ns	11.2	
	pН	4.95	5.06	_	4.92	5.02	6.97	ns	***	ns	0.091	
	Lactic acid	37.58	32.35	_	36.76	39.55	16.75	ns	*	ns	2.38	
	Acetic acid	17.06	12.07	_	18.07	16.68	7.81	ns	*	ns	1.17	
	Butyric acid	0.00	0.13	-	1.62	1.90	0.54	*	ns	ns	0.271	
	Yeast count	0.88	1.10	-	2.17	3.78	3.21	*	ns	ns	0.348	
	Mould count	2.45	2.10	-	3.41	2.86	6.54	*	**	ns	0.154	

<sup>1</sup>DM content (g kg<sup>-1</sup>); lactic, acetic, butyric acids (g kg<sup>-1</sup> DM); yeast and mould count (log cfu g<sup>-1</sup> of silage); F, film effect, Z, zone effect, F×Z interaction.

<sup>2</sup>No mould spots were present.

#### Conclusions

Data confirmed that four layers of a high oxygen barrier film could improve hygienic quality of baled silage compared to six layers of commercial PE film, by reducing mould development in two out of three trials at farm conditions. Furthermore, a high oxygen barrier film could reduce mould development even when the stretch film was damaged by bird puncturing.

#### References

Borreani G. and Tabacco E. (2008) New oxygen barrier stretch film enhances quality of alfalfa wrapped silage. *Agronomy Journal* 100, 942–948.

Borreani G. and Tabacco E. (2010) Use of new plastic stretch films with enhanced oxygen impermeability to wrap baled alfalfa silage. *Transactions of the ASABE* 53, 635–641.

Keller T., Nonn H. and Jeroch H. (1998) The effect of sealing and of additives on the fermentation characteristics and mould and yeast counts in stretch film wrapped big-bale lucerne silage. *Archives of Animal Nutrition* 51, 63–75.

Paillat J.M. and Gaillard F. (2001) Air-tightness of wrapped bales and resistance of polythene stretch film under tropical and temperate conditions. *Journal of Agricultural Engineering Research* 79, 15–22.

## Impact of sward properties on the predictability of forage quality and yield in grassland using remote sensing

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## Abstract

Remote sensing techniques have been gaining interest due to their potential for providing fast and non-destructive measurements of crop stands. We present the results of a field experiment aimed at determining the impact of sward structural parameters on the estimation of dry biomass yield and forage quality in grasslands based on spectrometric data. Using a portable spectrometer, we measured the red edge inflection point (REIP) of swards of different species composition at three dates following fertilizer application. We analysed the explanatory power of REIP, plant species, sward height and percent ground cover of green foliage for forage quality parameters and yield. We additionally analysed the effect of sward canopy structure on the predictability of yield for individual species. Our results indicate plant species to be an important factor for the prediction of both yield and forage quality. We also found evidence that biomass distribution across sward height layers and percent ground cover of green foliage are further factors to be considered for the estimation of grassland yield based on spectrometric measurement.

Keywords: spectrometry, crude protein, fibre content, sward structure, biomass distribution

## Introduction

Remote sensing techniques are of increasing importance as fast and non-destructive means of measuring characteristics of crop stands. For their successful application in grasslands, the optimisation of the interpretation of spectral signatures of multi-species swards and of stands with varying canopy architecture is of particular importance. Within the present experiment, we first tested the hypotheses that plant species, sward height and percentage ground cover of green foliage have an impact on the prediction of yield, as well as forage quality, based on the spectrometrically determined value of the red edge inflection point (REIP) (Guyot and Baret, 1988). Secondly, we tested the hypothesis that the accuracy of spectrometric yield measurement is dependent on sward canopy structure in terms of biomass distribution across height layers within the stand.

## Materials and methods

We used two mixed stands of grassland plants and thirteen monocultures, of which five were herbs and nine were grass species: *Achillea millefolium, Plantago major, Ranunculus repens, Taraxacum officinale, Agrostis stolonifera, Deschampsia cespitosa, Elymus repens, Festuca arundinacea, F. rubra rubra, F. trichophylla, Lolium perenne, Poa pratensis and P. supina.* The mixed stands were a grass-clover mixture of *L. perenne* and *Trifolium repens* with clover accounting for 5 to 80% of the yield, and a seed mixture comprising all of the mentioned fourteen species in equal proportions. The experimental design was a split-plot, with plant species as the main factor and fertilization as subordinate level (two levels: 20 kg N ha<sup>-1</sup> and 80 kg N ha<sup>-1</sup>), and with three replications grouped in randomized blocks.

Spectral measurements were made on a spring re-growth after 14, 28 and 54 growing days, using a portable field spectrometer (HandySpec<sup>VIS\_CT</sup>, Tec5 AG). Immediately after the spectral measurements, compressed sward height was determined with a rising plate meter, and digital photographs were taken to obtain a measure of the percent ground cover of green foliage by pixel count analysis. Herbage was cut in  $30 \times 30$  cm<sup>2</sup> subplots to 2 cm stubble height and its biomass determined after drying at 60°C. Dry herbage was ground and crude protein (CP), crude fibre (CF), and acid detergent fibre (ADF) content was estimated using near infrared reflectance spectroscopy (NIRS) (Kesting et al., 2009). For the determination of vegetation structure, additional samples were collected at the third measuring date by cutting an area of ca.  $7 \times 7$  cm<sup>2</sup> at ground level adjacent to each corner of the  $30 \times 30$  cm<sup>2</sup> subplots. These samples were divided into four fractions of sward-height layers: <5 cm above ground, 5 to 10 cm, 10 to 20 cm, and >20 cm above ground. Dry biomass yield was determined for all height layers progressing from the top towards the bottom of the sward, i.e. for the layers >20, >10, and >5 cm of height above ground level, and for the complete sward. Data were analysed using the software R, version 2.13.0 (R Development Core Team, 2011). Data were tested for variance homogeneity, and percent data were arcsine-square root transformed. Multiple linear regression models were formulated and validated to quantify the explanatory power of the factors REIP, plant species, percent cover of green foliage and sward height for the values of CP, CF, ADF, and for the dry weight of total harvested biomass for each of the three measuring dates. For the data on sward height layers, multiple linear regression models were formulated and validated for the individual species to analyse the correlation of the biomass of each layer with REIP, total sward height and percent cover of green foliage. For this data set, results are shown for A. millefolium, L.

perenne and P. pratensis.

#### Results

Linear regression analyses indicated plant species to be the most significant explanatory factor for the values of CP, CF and ADF, and its explanatory power increased for all of these parameters with later harvest dates. The spectral index of REIP was less important for explaining forage quality than the factor plant species, and its significance decreased with later harvest dates. Sward height had a low, yet significant explanatory power for forage quality. For the prediction of dry biomass yield, REIP had higher explanatory power than plant species. Percentage ground cover of green foliage generally was a variable of low significance for the explanation both of forage quality parameters and of yield (Table 1). The investigated species differed strongly in terms of sward structure. In A. millefolium, biomass was evenly distributed across the total height of the sward, whereas in L. perenne and in *P. pratensis* more than 80% of the total biomass was located within the height layer  $\leq 10$ cm above ground. These species also differed with regard to the linear regression models explaining biomass by REIP, sward height and percent ground cover of green foliage. In A. millefolium, total stand biomass could not accurately be predicted. The highest  $R^2$  values were obtained by correlating REIP with biomass of the sward layer >20 cm above ground level  $(R^2 = 0.92, P = 0.006)$ . In contrast, in *L. perenne* the best model to predict total stand biomass used REIP and total sward height as explanatory variables ( $R^2 = 0.90$ , P = 0.01). For P. pratensis, the best model to predict total stand biomass used REIP and percent ground

cover of green foliage as explanatory variables ( $R^2 = 0.92$ , P = 0.04). In the latter species, percent cover of green foliage averaged 47.9%, which is distinctly lower than that in *A*. *millefolium* (92.4%) and *L. perenne* (72.7%).

Table 1. ANCOVA table of multiple linear regression analysis of the response variables crude protein (CP), crude fibre (CF), acid detergent fibre (ADF) content and dry biomass (DM) of the swards with the explanatory variables REIP value, plant species (including 13 monocultures and two mixed stands), percent ground cover of green foliage, and sward height at three measuring dates.  $R^2$  values of models, F values, and explanatory power of explanatory variables (\*\*\*: P < 0.001, \*\* 0.001 < P< 0.01; \*: 0.01 < P < 0.05)

Mea	suring	RE	EIP (1 df)	spec	ies (14 df)	cover	green (1 df)	heig	ght (1 df)	$R^2$
d	ate	F	% var. expl.	F	% var. expl	F	% var. expl	F	% var. expl	
	CP	15.1	7.4***	7.4	50.9***	0.9	0.4	12.2	6.0***	0.56
$1^{st}$	CF	87.3	29.2***	9.0	42.2***	0.5	0.2	13.0	4.3***	0.70
	ADF	18.1	11.1***	3.9	33.0***	1.6	1.0	18.2	11.1***	0.46
	DM	23.3	18.2***	1.1	11.8	0.5	0.4	17.0	13.3***	0.30
	CP	33.6	13.8***	9.6	55.3***	2.7	1.1	0.04	0	0.63
$2^{nd}$	CF	24.9	6.3***	21.0	73.8***	0.9	0.2	6.3	1.6*	0.78
	ADF	1.0	0.5	10.4	66.2***	1.5	0.7	0	0	0.60
	DM	25.9	18.6***	2.2	21.7*	0.1	0.1	10.7	7.7**	0.36
	CP	6.8	1.8*	22.1	79.5***	0.6	0.2	0.4	0.1	0.77
$3^{rd}$	CF	75.8	12.8***	30.7	72.2***	2.8	0.5	14.5	2.4***	0.85
	ADF	0.7	0.3	13.0	68.5**	1.5	0.6	9.6	3.6**	0.67
	DM	174.4	45.5***	5.1	18.7***	4.9	1.3*	60.1	15.7***	0.77

#### **Discussion and conclusions**

Our results show that the factor plant species has a considerable impact on the predictability of forage quality parameters and yield based on spectrometric measurement of grassland. These results agree with Biewer *et al.* (2009) whose work on legumes indicates that species-specific calibrations may be required for spectrometric measurements of herbage yield. Sward height also proved to be a significant explanatory variable for the target parameters. The present data indicate that the distribution of biomass across sward height layers may be a determinant of the accuracy of yield estimation in grasslands. The question needs to be addressed as to whether in tall swards with biomass distributed evenly across the height of canopy, like in *A. millefolium*, spectrometric techniques may merely be able to measure the uppermost canopy layer. Another aspect which we perceive to be worth consideration for further research is the quantification of the impact of percent ground cover of green foliage on the estimation of yield based on spectrometric measurement and possibly the determination of threshold levels for this factor to influence the accuracy of measurement.

#### References

Biewer S., Fricke T. and Wachendorf M. (2009) Determination of dry matter yield from legume-grass swards by field spectroscopy. *Crop Science* 49, 1927–1936.

Guyot G. and Baret F. (1988) Utilisation de la haute résolution spectrale pour suivre l'état des couverts végétaux. In: Guyenne T.D. (ed) *Proceedings of the 4<sup>th</sup> International Colloquium on Spectral Signatures of Objects in Remote Sensing*, Aussois (Modane) France, 18-22 January 1988. European Space Agency Publications Division, Nordvijk, ESA SP 287, 279–286.

Kesting S., Wrage N. and Isselstein J. (2009) Herbage mass and nutritive value of herbage of extensively managed temperate grasslands along a gradient of shrub encroachment. *Grass and Forage Science* 64, 246–254. R Development Core Team (2011) R.A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

## Comparison of the agronomic effects of maturity and ploidy in perennial ryegrass

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#### Abstract

Perennial ryegrass varieties with increased herbage yield and nutritive value can lead to improved animal productivity and increase the efficiency of grass-based ruminant systems. Recently, national variety evaluation schemes have supplemented herbage yield data with nutritive quality traits. This study assessed the effects of maturity (early, intermediate and late) and ploidy (diploid and tetraploid) groups on herbage yield and three nutritive traits (*in vitro* dry matter digestibility, water-soluble carbohydrate and crude protein) of perennial ryegrass swards in a national variety evaluation scheme. Perennial ryegrass plots were sown at the Variety Evaluation Centre, Backweston, Ireland. Swards were managed under combined simulated grazing and conservation management scheme and harvested over three years. On an annual basis, higher digestibility (P < 0.001) and water-soluble carbohydrate concentration (P < 0.001) occurred for tetraploid compared to diploid swards and this was expressed in most seasonal periods. Later maturity was associated (P < 0.05) with higher annual herbage yields and crude protein concentration (P < 0.001) but this was not expressed in each seasonal period.

Keywords: perennial ryegrass, nutritive quality, digestibility, ploidy, maturity

## Introduction

Recommended List (RL) trials for perennial ryegrass evaluate varieties based on agronomic traits that are indicative of animal productivity (Grogan and Gilliland, 2010). Traditionally RL trials were primarily focused on yield and persistence however nutritive quality traits can have important economic implications (O'Donovan et al., 2010). Digestibility has been cited as the single best indicator of nutritional value (Casler 2000), with a 1% increase in grass digestibility resulting in an average 3.2% increase in live weight gain by beef cattle (Casler and Vogel, 1999). Miller et al. (2001) reported an additional 2.7 kg daily milk yield when fed a perennial ryegrass variety of increased water-soluble carbohydrate (WSC) concentration. This has lead to the Irish RL supplementing yield and persistence data with information on the DMD and WSC concentration. Within RL trials perennial ryegrass varieties are categorised into maturity (early, intermediate and late) and ploidy (diploid and tetraploid) groups that each have a niche role on farming enterprises. The objective of this study was to assess whether, despite significant differences between individual varieties, the maturity and ploidy classifications remain distinctive in their herbage yield potential and nutritive quality (in vitro DMD, WSC and crude protein(CP)) by examining data from the Irish Recommended List for Grass and Clover Varieties (DAFF, 2011).

## Materials and methods

Variety trials were carried out at the DAFM Variety Evaluation Centre, Co. Kildare Ireland. These were sown in three separate trials for maturity (early, intermediate and late) based on heading date. All varieties subject to evaluation were included in the model (n = 104). Within maturity groups, trials were in a randomised complete block design with 4 blocks. Plots ( $7.0 \times 1.5$  m) were sown in the year prior to first harvest at a sowing rate of 31 kg ha<sup>-1</sup> and 41 kg ha<sup>-1</sup> for diploids and tetraploids respectively. A split dressing of 350 kg N ha<sup>-1</sup> annum<sup>-1</sup> was applied in each harvest year. A 6 cut simulated grazing and conservation cutting scheme was carried out on the plots in the 2 years subsequent to sowing. Herbage yield was determined using a Haldrup harvester cut at 5 cm above ground level. A *c*.300g sample was collected from each plot and dried at 80°C for 16h to determine dry matter content. Each sample was subsequently milled (1 mm sieve) prior to nutritive analysis using near infrared reflectance spectroscopy. A REML analysis (Genstat) was carried out with maturity, ploidy and their interaction independently excluded from the full fixed model to assess their significance.

#### **Results and discussion**

The only significant interaction between maturity and ploidy in this study occurred for CP at 'Spring' (Table 1). The cause of the latter was difficult to determine as there was no difference in CP between the two ploidies or the three maturities. Maturity had an effect on the annual herbage yield (P < 0.05) with the only seasonal difference at Silage 1 (P < 0.01). The current results were in contrast to Gilliland *et al.* (2002) who found earlier groups to have a higher herbage yield in early April, corresponding to the 'Spring' period and the late group

		Herbage yield (t ha <sup>-1</sup> )						In vitro	DMD (g	kg <sup>-1</sup> )	
	Spring	Sil. 1	Sil. 2	ROY	Ann		Spring	Sil. 1	Sil. 2	ROY	Ann
Е	1.3	6.7	3.7	3.9	15.7	Е	854	702	802	813	767
Ι	1.1	6.5	4.4	4.2	16.3	Ι	853	765	793	798	789
L	1.2	6.3	4.1	4.5	16.0	L	846	792	798	803	800
SED	0.11	0.17	0.17	0.23	0.23	SED	9.7	19.2	3.0	1.9	9.6
D	1.3	6.6	3.9	4.2	15.9	D	843	746	797	798	780
Т	1.1	6.4	4.2	4.3	16.1	Т	859	760	798	811	791
SED	0.08	0.14	0.09	0.07	0.19	SED	1.5	4.1	2.5	1.6	2.5
Maturity	ns	**	ns	ns	*	Μ	ns	ns	**	***	ns
Ploidy	**	ns	***	ns	ns	Р	***	***	ns	***	***
MxP	ns	ns	ns	ns	ns	MxP	ns	ns	ns	ns	ns
		WSC i	n DM (g	kg <sup>-1</sup> )				CP in	DM (g k	$g^{-1})$	
	Spring	Sil. 1	Sil. 2	ROY	Ann	-	Spring	Sil. 1	Sil. 2	ROY	Ann
E	214	166	214	146	197	Е	180	96	125	133	118
Ι	212	205	201	149	202	Ι	183	102	120	144	122
L	226	208	204	155	208	L	182	113	120	140	125
SED	18.86	5.04	12.50	7.65	9.28	SED	7.71	8.35	3.93	5.96	1.55
D	202	183	202	145	195	D	181	104	123	134	123
Т	233	203	210	155	210	Т	182	103	121	137	121
SED	4.3	4.3	5.4	2.1	4.0	SED	1.4	1.99	1.7	1.5	1.3
М	ns	***	ns	ns	ns	Μ	ns	ns	ns	ns	***
Р	***	***	ns	***	***	Р	ns	ns	*	*	*
MxP	ns	ns	ns	ns	ns	MxP	***	ns	ns	ns	ns

Table 1. Seasonal and annual comparison of maturity and ploidy for yield and quality of perennial ryegrass

Sil. 1 – Silage 1, Sil. 2 – Silage 2, ROY – Rest of year. Ann. – Annual value. DMD – Dry matter digestibility, WSC – Water-soluble carbohydrate, CP – Crude protein. \*; \*\*; \*\*\*; N.S. stand for P < 0.05, P < 0.01, P < 0.001 and Non significant, respectively. E-; I-; L- – early, intermediate and late maturity groups. D – Diploid. T – Tetraploid. M – Maturity. P – Ploidy. SED – Standard error of difference. had a higher herbage yield in the summer period coinciding with the timing of the silage cuts of the current study. Diploid swards were higher yielding than tetraploid swards at 'Spring' (P < 0.01) though this relationship was reversed at 'Silage 2' (P < 0.001) resulting in no significant difference between ploidy groups over the growing season. This is in contrast to Gilliland et al. (2002) who found no seasonal differences between ploidy groups and tetraploids to be higher vielding than diploids across the growing season. Tetraploid swards had on average 11 g kg<sup>-1</sup>higher digestibility than diploid varieties (P < 0.001) across the growing season with this difference expressed at all seasonal periods except 'Silage 2'. Tetraploid varieties on average also had higher WSC concentration than diploid varieties (P < 0.001) and again this difference was pronounced at all season periods except 'Silage 2'. The increased DMD and WSC of tetraploids could be explained by an increased ratio of cell contents to cell wall in tetraploids that allows for increased intra-cellular components, such as WSC, without a proportional increase in potentially lower nutritive value cell wall material (Wilkins & Sabanci 1990) however tetraploid swards had a lower CP concentration than diploids on an annual basis (P < 0.05) and at the 'Silage 2' (P < 0.05) and 'Rest of Year' (P < 0.05) periods. Overall, seasonal differences between maturity groups were less frequent than between ploidies and displayed much less consistent advantage for any specific maturity.

#### Conclusions

The results indicate that breeding effort has been more successful in providing varieties with comparable yield and quality regardless of maturity than in overcoming the inherent differences between ploidies. Tetraploid swards had higher nutritive value in terms of DMD and WSC than diploids with no significant loss in annual yield, but a complete evaluation between these groups must take into consideration all agronomic factors including the lower persistence of tetraploids compared to diploids as reported on the Irish RL. Later maturity was associated with higher DMD and CP but weakly in individual seasonal periods. Although these differences are statistically significant, it is unclear as to the magnitude of difference that would provide increased animal performance, particularly with WSC (O'Donovan *et al.*, 2010). This uncertainty is a serious knowledge gap as O'Donavan *et al.* (2010) has identified quality characters as key factors for increasing profitability and efficiency of farm enterprises.

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#### References

Casler M.D. (2000) Breeding forage crops for increased nutritional value. *Advances in Agronomy* 71, 51–107. Casler M.D. and Vogel K.P. (1999) Accomplishments and impact from breeding for increased forage nutritional value. *Crop Science* 39, 12–20.

Grogan D. and Gilliland T.J. (2010) A review of perennial ryegrass variety evaluation in Ireland. In: O'Donovan M., and Hennessy D. (eds) *Grasses for the Future*. Teagasc, Co. Cork, Ireland, pp. 99–118.

Gilliland T.J., Barrett P.D., Mann R.L., Agnew R.E. and Fearon A.M. (2002) Canopy morphology and nutritional quality traits as potential grazing value indicators for *Lolium perenne* varieties. *Journal of Agricultural Science* 139, 257–273.

Miller L.A., Moorby J.M., Davies D.R., Humphreys M.O., Scollan N.D., MacRae J.C. and Theodorou M.K. (2001) Increased concentration of water-soluble carbohydrate in perennial ryegrass *Lolium perenne*: milk production from late-lactation dairy cows. *Grass and Forage Science* 56, 383–394.

O'Donovan M., Lewis E., Boland T. and O'Kiely P. (2010) Requirements of future grass based ruminant production systems in Ireland. In: O'Donovan M., and Hennessy D. (eds) *Grasses for the Future*. Teagasc, Co. Cork, Ireland, pp. 11–42.

Wilkins P.W. and Sabanci C.O. (1990). Genetic variation in leaf epidermal cell size and shape in *Lolium perenne*. *Euphytica* 47, 233–239.

## Impact of development stage, wilting and addition of ground corn on the quality and nutritional value of sainfoin (*Onobrychis viciifolia*) silage

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## Abstract

The results of an experiment with ensiling sainfoin at different stages of development with ground corn are presented in this paper. Sainfoin biomass was cut at two stages (early flowering (10.05.2010) and full flowering (01.06.2010)), and ensiled fresh or after wilting, without additives and with the addition of 3% and 6% of ground corn. In the early flowering stage of sainfoin, the, biomass was better in terms of its floristic composition due to a lower presence of other plant species (the proportion of sainfoin was 85.49% at the earlier flowering stage and 76.255 at full flowering stage), with a more favourable ratio of leaf, leaf stems and stems compared to the full-flowering stage. Cutting at the later stage contributed to the significantly higher dry matter content of silage compared with the earlier stage (357.4:247.4 g kg<sup>-1</sup>), which contributed to the lower acidity of the silage from the later stage (pH 4.40:4.22), and also less production of butyric acid. Wilting, especially in the early flowering stage, contributed to the higher quality of silage with a lower ratio of butyric acid. The proportion of lactic acid in all silages was favourable compared to that of acetic and butyric acid. Addition of ground corn to sainfoin biomass, especially at the earlier growth stage, provided a slightly better fermentation and lower pH value.

Keywords: sainfoin, stage, wilting, ground corn, pH

## Introduction

Sainfoin (*Onobrychis viciifolia*) is a perennial legume prevalent in eastern Serbia as a forage crop. However, regardless of the small land surface, it provides a good herbage yield at the first cut, and is of very good quality, with content of crude protein (CP) close to 20% (19.78%) of the dry matter (DM), and favourable content of crude fibre 25% (Tomić *et al.*, 2005). The aim of this study is to present the significance of the plant development stage for the quality of sainfoin silages, considering chemical composition and nutrient value and the achievement of satisfactory levels of fermentation.

## Materials and methods

Sainfoin biomass obtained at the first cut was ensiled according to a three-factorial experimental design  $(2 \times 2 \times 3)$  with three replicates. Factors were as follows: first, factor A – stage of plant development, two levels  $(a_1 - \text{cut on 10 May} \text{ and } a_2 - \text{cut on 1 June 2010})$ ; second factor B – dry matter, two levels  $(b_1 - \text{non-wilted biomass}, b_2 - \text{wilted biomass})$ ; and third factor C – additives  $(c_1 - \text{no additives}, c_2 - \text{with added 3% of ground corn, and } c_3 - \text{with ground corn, 6%})$ . Ensiling was carried out in experimental pots of volume of 120 litres. Sainfoin was first cut on 10 May, at the beginning of flowering stage, and the second cut was carried out on 1 June at the pod-formation stage. Dry matter was determined by drying at 65°C. Chemical analyses were carried out in the laboratory of the Institute for Forage Crops in Kruševac, according to standard methods (AOAC, 1990). The following parameters were determined: DM, CP, crude fibre (CF), crude fat, ash, Ca and P. Nutritional value was

expressed in NE<sub>L</sub> and NE<sub>M</sub> units, calculated according to the method of Obračević (1990), and nutrient digestibility coefficients according to Đorđević *et al.* (2003). The acidity (pH), the amount of ammonia nitrogen and lower fatty acids (acetic, butyric and lactic) were determined in the silage. For assessment of the success and quality of conservation, the DLG method was applied (Đorđević and Dinić, 2003). The results of chemical analyses were processed using variance analysis (Statistica 11, StatSoft Inc.), and statistical significance was tested using LSD-test.

#### **Results and discussion**

The silage was opened and the following sensory (organoleptic) observations were registered: odour was unpleasant in silages made from non-wilted biomass from the first growth stage, especially without additives; the structure was compromised, slimy, mucous, with high moisture; and the colour was dark olive. Effluents were present at the bottom of the experimental dish. High statistical significance for the content of dry matter for all three study factors was established (stage of development, level of dry matter and carbon hydrate additive).

Table 1. Results of chemical analyses of the fermentation processes and parameters of sainfoin silage quality (g kg<sup>-1</sup>DM)

Treatments	DM gkg <sup>-1</sup>	pН	А	В	L	СР	CF	Са	Р
a <sub>1</sub> b <sub>1</sub> c <sub>1</sub>	171.7	5.38	57.7	17.3	78.8	176.0	345.7	10.6	3.0
$a_1b_1c_2$	183.3	4.51	50.1	7.9	97.0	146.1	346.6	8.7	2.8
$a_1b_1c_3$	228.7	4.00	77.5	0.9	73.9	153.6	369.9	10.5	2.8
$a_1b_2c_1$	276.7	4.43	64.0	0.0	99.3	182.1	351.5	11.5	3.2
$a_1b_2c_2$	305.3	4.35	46.0	2.1	109.1	177.9	303.0	9.0	3.46
$a_1b_2c_3$	320.0	4.22	32.2	2.7	108.4	174.6	286.7	7.8	3.3
Average, a	247.4	4.48	54.6	5.1	94.4	168.6	333.9	9.68	3.10
$a_2b_1c_1$	282.3	4.42	43.7	15.3	103.4	137.8	413.7	7.3	2.6
$a_2b_1c_2$	336.6	4.35	25.5	4.6	99.7	158.8	352.4	8.9	2.7
$a_2b_1c_3$	351.0	4.24	33.4	4.5	99.1	148.2	334.6	9.2	2.76
$a_2b_2c_1$	371.6	4.46	37.1	0.0	89.2	148.7	356.5	7.8	3.1
$a_{2}b_{2}c_{2}$	393.3	4.48	38.6	0.8	96.9	154.6	358.8	8.0	2.66
a,b,c,	410.0	4.47	23.7	0.0	108.5	143.4	392.2	12.0	2.66
Average, a,	357.4	4.40	33.6	4.2	99.4	148.6	368.0	8.88	2.75
LSD 005 A B	7.2	0.20	7.9	2.4	7.9	4.97	25.7	0.72	0.16
LSD	8.7	0.25	9.7	3.0	9.7	6.09	31.5	0.88	0.21
LSD 001 A B	9.7	0.28	10.7	3.3	10.7	6.74	34.8	0.97	0.23
LSD 0.01 C	11.9	0.34	13.1	4.0	13.1	8.25	42.6	1.19	0.28

Legend: DM – dry matter, pH – acidity level, A – acetic acid, B – butyric acid, L – lactic acid, CP – crude proteins, CF – crude fibres, Ca – calcium, P – phosphorus.

The least favourable pH 5.38 value was established in silages made from non-wilted herbage at the first growth stage without additives (Table 1). Relative content of acetic acid in the total acids ranged from 17.93% ( $a_2b_2c_3$ ) to 50.89 ( $a_1b_1c_3$ ) (Table 2). A very significant effect on content of butyric acid was established for wilting of biomass and addition of ground corn, which confirmed the claim that butyric acid bacteria reduce their activity with the increase of dry matter content in silage. Wilting had a significant effect on production of lactic acid in silage made of plants harvested at the first stage of development. Content of nutrients (protein and fibre) was significantly better in sainfoin silages made from biomass harvested at the first stage of development. Content of proteins was 20 g kg<sup>-1</sup>DM higher in silages obtained from biomass cut at the flowering stage, and in contrast, content of crude fibre was lower 34 g kg<sup>-1</sup>DM. Concentrations of Ca and P were higher in silages made using plants in the first stage. Content of Ca was significantly affected by addition of ground corn, whereas the content of P was under significant effect of wilting. In the assessment of the quality of silage according to DLG method, it was established that the least score, of a possible 50, was for the silage made of non-wilted biomass cut at the stage of beginning of flowering without use of additives (treatment  $a_1b_1c_1$ ; 33 points – third class), and the highest score (48 points – first class) was obtained for silages from the following 4 treatments  $(a_1b_2c_2, a_1b_2c_3, a_2b_2c_1, a_2b_2c_3)$ . It was observed that increased level of dry matter contributes to improvement of the quality of silage, i.e. silages from the first stage were scored by three points less than silages from the second stage (Table 2).

Table 2. Share of acetic, butyric and lactic acids in total acids, expressed in %, and silage scores according to DLG method

Treatments	TA	SA	SB	SL	POINTS	CLASS
$a_1b_1c_1$	15.38	37.52	11.27	51.23	33	III
$a_1b_1c_2$	15.50	32.32	5.09	62.59	44	Ι
$a_1b_1c_3$	15.23	50.89	0.59	48.52	37	II
$a_1b_2c_1$	16.33	39.19	0.00	60.81	45	Ι
$a_{1}b_{2}c_{2}$	15.72	29.26	1.34	69.40	48	Ι
$a_1b_2c_3$	14.33	22.47	1.88	75.65	48	Ι
Average, a	15.41	35.43	3.31	61.26	44	Ι
$a_2b_1c_1$	16.24	26.91	9.42	63.67	44	Ι
$a_2b_1c_2$	12.98	19.63	3.55	76.82	47	Ι
$a_{2}b_{1}c_{3}$	13.70	24.38	3.28	72.34	47	Ι
$a_2b_2c_1$	12.63	29.37	0.00	70.63	48	Ι
a,b,c,	13.64	28.30	0.62	71.08	47	Ι
a,b,c,	13.22	17.93	0.00	82.07	48	Ι
Average, a.	13.72	24.49	3.06	72.45	47	Ι

Legend: TA – total acids (%), SA – share of acetic acid, SB – share of butyric acid, SL – share of lactic acid.

#### Conclusions

Considering the chemical results obtained for sainfoin silage, we can conclude that silages made from sainfoin herbage harvested at the beginning of flowering have a more favourable chemical composition in terms of crude protein quantity, fibre, Ca and P, but, due to increased moisture, ensiling of such biomass is difficult. In contrast, silages obtained from sainfoin cut at the pod-formation stage have significantly less crude protein, high level of fibre, and a better fermentation process because of higher content of dry matter and relative share of acetic, butyric and lactic acids in total acids. If young plants are ensiled at a significantly lower dry matter content, wilting of biomass is recommended, as well as adding of carbon hydrate additives to increase the dry matter content and create optimal conditions for activity of lactic acid bacteria.

## References

AOAC (1990) Official Methods of Analysis, 14th Edn., Association of Official Analytical Chemists, Washington D C. Arlington, Virginia, USA.

Đorđević N. and Dinić B. (2003) *Siliranje Leguminoza*, Institut za istraživanja u poljoprivredi. Beograd. Đorđević N., Grubić G. and Jokić Ž. (2003) *Osnovi ishrane domaćih životinja – Praktikum*. Univerzitet u Beogradu, Poljoprivredni Fakultet.

Obračević Č. (1990) Tablice hranljivih vrednosti stočnih hraniva I normative u ishrani preživara. Naučna knjiga, Beograd.

Tomić Z., Nešić Z., Krnjaja V. and Žujović M. (2005) Parameters of production and quality of new cultivars of perennial leguminous plants as livestock feed. *Biotechnology in Animal Husbandry* 21(3-4), 89–96.

# Biomass and protein yield of barley in response to different fertilization treatments in a semi-arid climate

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## Abstract

An experiment was established in a semi-arid Mediterranean environment, to determine dry matter (DM) and protein yields of barley forage influenced by different fertilization treatments. Five treatments were compared: unfertilized, and two doses of NPK combined with the addition of two different mycorrhizal inoculants. The results showed that the addition of fertilizer significantly increased DM biomass and protein yields of barley at medium-milk phenological stage. The differences in the response between the two doses of mineral fertilizer combined with the microorganism were not statistically significant, although the highest doses caused a slight increase in biomass and protein production. Furthermore, the fertilization treatment did not significantly affect protein concentration.

Keywords: Hordeum vulgare, yield, protein content, fertilization, microorganisms

## Introduction

Barley (*Hordeum vulgare* L.) has a wide variety of uses, including its usage for animal feed as whole crop forage, usually associated with a legume, or as part of livestock diets in the form of feed concentrate.

Chemical nitrogen fertilizers increase forage and grain production, but excessive use can lead to environmental problems, such as groundwater contamination. In addition, the cost of this type of fertilizers has increased, so there are new alternatives such as bio-fertilizers, which use microorganisms in the basis of their potential to promote plant nutrition, and to protect plants against soil-borne pathogens simultaneously (Johansen *et al.*, 1994; Guerra Sierra, 2008). The objective of this work was to evaluate the effect of mycorrhizal inoculants associated with mineral fertilization on biomass and protein production of barley crop without irrigation, in a semi-arid climate.

## Materials and methods

An experimental plot with a two-row barley variety (Volley) was established in the experimental field of the IRNASA-CSIC (Salamanca, Spain) in autumn 2010, without irrigation. The climate corresponds to a semi-arid Mediterranean type with mean monthly temperatures of 2.1°C minimum and 31.0°C maximum and total average annual precipitation of 452 mm. The soil type was a clay loam chromic luvisol with an acid pH (5.1).

The experimental design consisted of 5 subplots  $(200 \times 12 \text{ m}^2)$  corresponding to each of the five fertilizers tested. The subplots were separated by a passage of 0.20 m. Each subplot was divided into 4 zones considered as replicates of each treatment. Two doses of mineral fertilizer were considered: a) F1 = 100 kg ha<sup>-1</sup> of NPK 10-5-20 applied at sowing and 400 kg ha<sup>-1</sup> of a mixed nitrogenous fertilizer (27% N, 29% SO<sub>3</sub>) top dressing, and b) F2 = 300 kg ha<sup>-1</sup> of NPK 10-5-20 applied at sowing and 400 kg ha<sup>-1</sup> of the same top fertilizer. As inoculants, two commercial powder formulations were considered: a) M1 inoculant with spores of *Glomus intraradices* 

and *G. mosseae*, and b) M2 inoculant with five species, *G. intraradices*, *G. mosseae*, *G. claroideum*, *G. etunicatum* and *G. microaggregatum*, with a greater concentration of spores than M1. Each mineral treatment (F1 and F2) was combined with each mycorrhizal inoculant (M1 and M2). A F0 treatment without fertilizer addition was also considered. The inoculant addition was made directly to the seed, just before sowing.

Plants were harvested at medium-milk phenological stage, by cutting above-ground biomass at 2 cm above ground level. Dry biomass production, protein concentration (N Kjeldahl  $\times$  6.25) and protein production per unit area were determined. Data were statistically analysed using analysis of variance (ANOVA), and least significant difference (LSD) test was used for comparison of means (SPSS statistics 19).

#### **Results and discussion**

Mineral fertilization applied together with mycorrhizal inoculants significantly increased (P < 0.05) dry matter production of barley forage compared to the unfertilized control (Table 1). The yield obtained with the highest input of mineral fertilizer and M2 mycorrhiza (F2M2 = 711 g m<sup>-2</sup>) quadrupled that of the control (F0M0 = 179 g m<sup>-2</sup>). Considering the same dose of mineral fertilizer, the M2 mycorrhiza had a trend for higher biomass production, possibly due to the application of a greater mycorrhizal species number and spore concentration. This suggests that the M2 inoculant has a potential benefit to the barley crop. The application of M2 would decrease the mineral fertilizer input, thus lowering costs and contributing to a greater environmental protection. Garate and Bonilla (2001) indicated the positive effect of mycorrhizal inoculation on plant growth, although other authors claim that high inputs of mineral fertilizer can sometimes inhibit the effect of inoculants (Gianinazzi and Schüepp, 1994).

Regarding the protein concentration, there were no significant differences (P > 0.05) between treatments (Table 1). The increase in the nitrogenous fertilization level increased biomass production and this dilution effect hampered an increase in the N concentration. Moreover, protein concentration in those treatments including M2 tended to be higher than those with M1, which was similar to the effect on biomass production.

Protein production, resulting from the extraction of N from soil and biomass production, followed a similar trend to that of biomass production (Table 1). There were significant differences (P < 0.05) between F0 and the other treatments. The effect of the application level on protein production was greater than on biomass production. As a result, protein production with the highest level of fertilizer was 4.5 times higher than in F0 (63.0 g m<sup>-2</sup> vs. 14.1 g m<sup>-2</sup>). The effect of F2M2 on protein production was significantly higher than the treatment with F2M1, which indicates the positive effect of M2. Our results agree with those of Garate and Bonilla (2001) which indicated the positive effect of

Table 1. Effect of the fertilizer treatment on the biomass and protein yield and protein content of barley forage

Treatment	Yield	d (g m <sup>-2</sup> )		Protein co	ontent (g k	(g <sup>-1</sup> )	Protein yield (g m <sup>-2</sup> )			
Treatment	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	
F0	106-242	179 <sup>a</sup>	56.6	62.2-89.0	80.9 <sup>a</sup>	12.61	9.37-17.4	14.1 <sup>a</sup>	3.39	
F1M1	492-600	534 ь	46.2	75.0-82.2	78.0 a	3.10	48.0-59.5	53.7 <sup>b</sup>	5.39	
F1M2	564-700	609 <sup>bc</sup>	61.7	77.2–98.7	87.8 a	10.5	47.8-57.2	53.1 <sup>b</sup>	3.91	
F2M1	548-712	655 °	73.0	75.1-88.9	83.3 ª	6.00	48.7–58.4	54.3 <sup>b</sup>	4.26	
F2M2	552-828	711 °	115	84.9-96.2	89.1 a	5.00	53.1-70.3	63.0 °	7.21	
Total	106-828	538	204	62.2–98.7	83.8	8.48	9.37-70.3	47.6	18.2	

F0 = without fertilizer; F1, F2 = mineral fertilizer; M1, M2 = mycorrhiza. Different superscripts a, b, c indicate significant (P < 0.05) differences between fertilization treatments.

mycorrhizae in enhancing the capacity of the root to absorb certain ions such as phosphate, ammonium, nitrate and potassium.

#### Conclusions

The addition of mineral fertilizer combined with mycorrhizal inoculants significantly increased biomass and protein production. A positive effect of the mycorrhizal inoculant with a greater number of fungal species and spore concentration was observed.

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#### References

Gárate A. and Bonilla I. (2000) Nutrición mineral y producción vegetal. In: Azcón Bieto J. and Talón M. (eds) *Fundamentos de fisiología vegetal*, McGraw-Hill/Interamericana de España, Barcelona, Spain, pp. 113–130.

Gianinazzi S. and Schüepp H. (1994) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems, Birkhäuser Verlag, Basel, Switzerland, 226 pp.

Guerra Sierra B.E. (2008) Micorriza arbuscular. Recurso microbiológico en la agricultura sostenible. *Tec-nología en marcha* 21,191–201.

Johansen A., Jacobsen I. and Jensen E.S. (1994) Hyphal N transport by a vesicular-arbuscular mycorrhizal fungus associated with cucumber grown at three nitrogen levels. *Plant and Soil* 160, 1–9.

# **Differential** responses to timing of defoliation in perennial ryegrass (*Lolium perenne* L.) grown under silage management

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## Abstract

Plant breeders and testing authorities place increasing importance on enhancing herbage quality but some evidence exists that management interactions can alter evaluations. This study compared yield and herbage quality (digestibility, D-value; neutral-detergent fibre, NDF; acid-detergent lignin, ADL) in perennial ryegrass when test protocols differed in the timing of the first silage cut by up to 28 days, with a fixed regrowth of six weeks to the second cut. Increasing and decreasing trends in yield and quality with delayed cut were largely reversed at the second cut for all but ADL, which had a more novel response pattern. The effects of timing of defoliation were shown to be greatly reduced or eliminated by combining the first and second silage cuts and offered a better assessment of genetic potential, except for ADL. These findings also have implications for cultivar evaluation programmes and pose questions regarding ryegrass physiology relating to the observed differential responses in herbage quality parameters.

Keywords: grassland, differential response, cut date, D-value, NDF, ADL

## Introduction

Perennial ryegrass (*Lolium perenne* L.) is the most important agricultural sown grassland species in the UK and Western Europe. Agriculture in N. Ireland is predominantly grass based, playing a vital role in food sustainability and security; silage accounts for over 98% of overall conserved grassland production (Anon, 2010). Forage breeders' selection strategies and UK National List evaluation trials annually classify new improved cultivars for agriculture. Results reported by Hoppé and Gilliland (2010) address the response of ryegrass to cultivar maturity and cut date on the rank order of cultivars. For grass-based agriculture to meet the increasing demands of production, quality and environmental protection, plant breeders and official testing authorities will have to evaluate new cultivars in field trials under silage and simulated grazing management. In addition, there is a need to better understand the differential responses for yield and herbage quality that can be induced by the testing system and may obscure true genetic differences. This study examines the effects of delayed timing of defoliation on the yield and quality of perennial ryegrass at the first silage cut and the implications for the following cut.

## Materials and methods

A randomized split plot experiment, with five intermediate diploid cultivars differing in mean heading date was established with three replicates in July 2009 at Loughgall, County Armagh, as described by Hoppé and Gilliland (2010). Treatments were assessed under a 2-cut silage management, with 5 'harvesting cycles' (S-2, S-1, S0 [target 50% ear emergence], S+1, S+2) at 7-day intervals (28-day span) commencing with S-2 on 13 May 2010. Each first cut was given a regrowth period of 6 weeks to cut 2 (S-2 cut date 24 June). A compound fertiliser of 80 kg N ha<sup>-1</sup> was applied after each harvest. All treatments were harvested in 2010 for herbage dry matter (DM) yield and herbage quality expressed as D-value, Neutral detergent fibre (NDF) and acid detergent lignin (ADL) determined using Near Infra-Red Spectroscopy (Archer – personal communication). All plot data (cultivar means) for silage cuts 1 and 2 and combined analyses were subjected to analysis of variance using GENSTAT (Payne *et al.*, 2011).

#### Results

The effect of delaying the timing of the first silage cut induced highly significant (P < 0.001or P < 0.01) responses at cut 1 and cut 2 in all measured parameters (Table 1). However, these responses to timing were different for each parameter. The DM yield increased with delaying defoliation at cut 1, rising progressively from the earliest defoliation date (S-2, 1.81 Mg ha<sup>-1</sup>) to a maximum at the latest defoliation date  $(S+2, 8.10 \text{ Mg ha}^{-1})$ . The cut 2 yields, which were taken six weeks after each of the first defoliations, displayed a reversed trend with a 'step-wise' reduction from 8.55 Mg (S-2) to 5.58 Mg (S0) and 3.1 Mg at S+2. The overall effect of this reversal was that there was a reduced significance (P < 0.05) in yield response to the timing treatment when the combined cut 1+2 yields were calculated. These combined yield values did, however, display a 'residual' trend from S-1, at 4.63 Mg to S+2 at 5.60 Mg. The delayed cutting treatment induced a declining D-value from S-2 to S+2 which was not entirely reversed in the second cutting cycle as there was a significant rise from S-2, S-1 to S0, but treatments S0, S-1 and S-2 were not different and indeed in the same numerical order as the first cut. This resulted in a small significant difference remaining in the combined cut 1+2 average, for the S+2 treatment (64.7%) and other S treatments (range 67.4 to 69.4%).

Variable	Variable Silage cut <u>'Harvesting Cycle'</u>						ISD	Sig
variable	number	S-2	S-1	S0	S+1	S+2	LSD	Sig.
DM Yield	1	1.81	3.68	5.17	6.29	8.10	0.81	***
(Mg ha <sup>-1</sup> )	2	8.55	5.58	5.50	4.88	3.10	1.00	***
-	1+2	5.18	4.63	5.33	5.59	5.60	1.18	*
D-value	1	79.8	72.7	68.3	65.7	61.2	3.67	***
(%)	2	58.8	65.2	70.0	69.1	68.2	3.92	***
	1+2	69.4	68.9	69.2	67.4	64.7	2.73	*
NDF	1	43.9	47.8	49.2	50.7	50.6	2.99	***
(% of DM)	2	53.7	50.3	45.7	46.4	45.8	3.48	**
	1+2	48.8	49.1	47.5	48.6	48.2	2.46	NS
ADL	1	3.07	4.12	5.33	5.23	4.60	0.41	***
(% of DM)	2	4.56	4.33	4.43	4.49	3.74	0.29	***
	1+2	3.82	4.23	4.88	4.86	4.17	0.30	***

Table 1. Mean dry matter (DM) yield (Mg  $ha^{-1}$ ) and chemical composition for perennial ryegrass silage cuts 1, 2 and combined cut (1+2) for five harvest cycles (S treatments) in 2010

LSD, least significant difference (P < 0.005); NDF, neutral-detergent fibre; ADL, acid-detergent lignin.

The pattern of responses in the NDF contents was, as would be expected, broadly consistent with that of the overall digestibility measurement of D-value. Increasing fibre and declining digestibility occurred from S-2 to S+2 at the first cut, though significance was only achieved between S-2 and S-1. At the second cut, the same reversal pattern occurred as with D-value, as significant differences existed from S-2 to S0, with S0, S1 and S-2 not differing significantly. This reversal of responses at first and second cuts completely removed any evidence of treatment effects when the combined cut 1+2 was calculated. Finally, ADL displayed

a different response pattern to delayed timing than recorded in the other parameters. At cut 1, the earliest timed defoliation (S-2) was significantly lower while S0 and S-1 were significantly higher than S+1 and S+2. At the second cuts, there was a progressive rise across all five treatments, from S+2 to S-2. This resulted in highly significant differences remaining between treatments when these two cuts were amalgamated in the combined cut 1+2.

## Discussion

There is an emphasis by plant breeders and testing authorities on the importance of enhancing cultivar herbage quality but as Gilliland *et al.* (2005) showed, grass variety  $\times$  management interactions may lead to incorrect evaluations. The observed reverse responses in the current study to delayed timing in yield and D-value were similar to that reported by Aldrich and Dent (1967) and Gilliland *et al.* (1995). These authors observed that delaying the first cut increased and decreased silage cut 1 and 2, respectively. Their conclusion, that the increasing maturity of the ryegrass as the first cut was delayed caused a slower recovery in the following fixed regrowth period, therefore remains appropriate to the current study. This would explain both the lower yields and higher quality observed at the second cut. The dissimilarities in the timing responses of the NDF fraction show evidence of threshold levels having been reached and differential responses into the second cuts in the fibre fractions that comprise the overall digestibility. This was further confirmed by the ADL responses which did not synchronise with the other parameters. Moreover, while combining the two cuts removed or greatly decreased the treatment effect in these parameters, this did not happen for ADL.

## Conclusions

The study has implications for cultivar evaluation trials. An important finding was that combined mean silage yield was less affected by delayed cutting and would appear to closer reflect the grass genetic potential by removing confounding effects of trial management. The responses of quality parameters indicate differential physiological reactions in perennial ryegrass to timing of defoliation that merits further investigation.

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## References

Aldrich D.T.A. and Dent J.W. (1967) The relationship between yield and digestibility in the primary growth of nine grass varieties. *Journal of the National Institute of Agricultural Botany* 11, 104–113.

Anon (2010) Crop Production – Statistical Review of Northern Ireland Agriculture 2010. *Department of Agriculture and Rural Development for Northern Ireland*. A National Statistics Publication, pp. 32.

Gilliland T.J., Camlin M.S. and Johnston J. (1995) Effect of harvest date and cultivar maturity on perennial ryegrass (*Lolium perenne*) yield and digestibility. *Irish Journal of Agricultural and Food Research* 34, 133–142. Gilliland T.J., Agnew R.E., Fearon A.M. and Wilson F.E.A. (2005) Perennial ryegrass variety differences in nutritive value characteristics. In: O'Mara F. *et al.* (eds) *XX International Grassland Congress, Dublin, offered papers*, p. 175. Wageningen NL, Wageningen Academic Publishers.

Hoppe G.M. and Gilliland T.J. (2010) Implications of cultivar maturity and time of cutting for testing and breeding programmes – a potential problem? 10<sup>th</sup> Research Conference Proceedings, British Grassland Society, Belfast, September 2010, pp 10–11.

Payne R.W., Harding S.A., Murray D.A., Soutar D.M., Baird D.B., Glaser A.I., Welham S.J., Gilmour A.R., Thompson R. and Webster R. (2011) *The Guide to GenStat Release 14, Part 2: Statistics.* VSN International, Hemel Hempstead, UK.

# New modules for the simulation of timothy yield and nutritive value: regrowth and harvest index

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#### Abstract

The grass model CATIMO was initially developed to simulate the growth and nutritive value of the primary growth of timothy (*Phleum pratense* L.). New modules were developed to simulate the growth and nutritive value of the regrowth, and the dynamics of the harvest index. The regrowth was simulated through reserve–dependent growth and the remobilization of carbohydrate and N reserves. New functions of shoot apex height and plant weight density were used for the simulation of the harvest index. These new modules were integrated into CATIMO, which was then assessed on leaf area index (LAI), above-ground biomass and forage dry matter (DM) yields, three attributes of nutritive value, and harvest index, with data from field experiments in Norway, Finland and Canada. The regrowth DM yield of timothy was simulated satisfactorily with a similar predictive ability as in the primary growth, but the simulation of the regrowth nutritive value was less successful. The harvest index was successfully predicted. The modified CATIMO model provides a framework to explore options for high yield with high nutritive value.

Keywords: carbohydrate, nitrogen, reserve-dependent, thermal time

#### Introduction

The grass model CATIMO was initially developed to simulate the growth, dry matter (DM) yield, and nutritive value of the primary growth of timothy (Bonesmo and Bélanger, 2002a,b). Few models simulate the regrowth of grasses (Höglind *et al.*, 2001; Ruget *et al.*, 2009) but none have the ability to simulate the nutritive value of the regrowth and the harvest index. Our objectives were (i) to further develop CATIMO by adding the simulation of the growth and nutritive value of the regrowth and the harvest index, and (ii) to evaluate the modified model using experimental data from different environments.

#### Materials and methods

The regrowth was simulated through reserve–dependent growth with stored carbohydrates and N flowing into new meristems for regrowth. The reserve–dependent growth had a growth efficiency ( $Y_G$ ) of 0.75 g DM g<sup>-1</sup> reserves (Thornley and Cannell, 2000). The rate of reserve–dependent growth [ $dRDG/dT_r$ , g DM m<sup>-2</sup> (°C–d)<sup>-1</sup>] for timothy was quantified as follows:

$$\frac{dRDG}{dT_t} = R_{RGD} \cdot (RDG_{\max} - RDG) \cdot Y_G$$

where *RDG* is the reserve–dependent growth (g DM m<sup>-2</sup>),  $T_i$  is the daily active temperature above base temperature (°C–d),  $R_{RDG}$  is the relative growth rate [(°C–d)<sup>-1</sup>], and  $RDG_{max}$  (g DM m<sup>-2</sup>) is the maximum use of stored reserves corresponding to the amount of reserves at the start of regrowth. The biomass from reserve–dependent growth was partitioned into leaves to calculate the initial leaf area index (LAI) through the specific leaf area.

At the beginning of regrowth, the nutritive-value attributes were reinitialized by setting the NDF concentration to a minimum value and the digestibility of the NDF (dNDF) to a maximum value for leaves and stems; the initial N concentration depended on accumulated N and biomass. A new module to simulate the forage harvest index was added to CATIMO using a novel approach based on plant weight density (DM yield per unit of shoot height) and shoot apex height (Jing *et al.*, 2011).

Regrowth simulations were evaluated with data from four field experiments conducted under rainfed conditions in Norway, Finland, and western and eastern Canada (Höglind *et al.*, 2005; Bélanger *et al.*, 2008; Virkajärvi *et al.*, 2011). Simulations of the harvest index were evaluated with data of the primary growth from experiments in Canada (Jing *et al.*, 2011). The model default parameters have been presented previously (Bonesmo and Bélanger, 2002a,b; Jing *et al.*, 2011) and the model evaluation approach is described in Jing *et al.* (2011).

#### **Results and discussion**

*Regrowth biomass and LAI.* Above-ground biomass and forage DM yields of the regrowth were simulated satisfactorily in both calibration and validation sets, with normalized root mean square errors (NRMSE, 15–27%) between simulated and measured values and model simulation efficiency (EF, 0.78–0.94) comparable to those of the primary growth (Table 1). The NRMSEs of simulated LAI of the regrowth were high (>40%) and slightly greater than those of the primary growth, but LAI values under 4.0 were successfully simulated. Our approach for simulating regrowth differs from that of LINGRA where leaf appearance is correlated to the concentration of carbohydrate reserves (Höglind *et al.*, 2001) and that of STICS in which LAI is initialized at the beginning of regrowth (Ruget *et al.*, 2009). Our results confirm that an approach based on reserve–dependent growth can successfully simulate the regrowth of grasses.

Crop attribute		Primary	growth		Regro	owth
Crop autibule	Ν	EF	NRMSE (%)	Ν	EF	NRMSE (%)
Calibration set						
Leaf area index	19	0.35	25	22	0.40	44
Above-ground biomass (g DM m <sup>-2</sup> )	11	0.94	15	11	0.94	15
Forage DM yield (g DM m <sup>-2</sup> )	35	0.79	15	26	0.87	26
N concentration (g kg <sup>-1</sup> DM)	35	0.61	17	26	0.08	19
NDF (g kg <sup>-1</sup> DM)	35	-0.01	9	26	-0.32	12
dNDF (g kg <sup>-1</sup> NDF)	25	0.64	7	17	0.66	5
Validation set						
Leaf area index	29	0.69	37	44	0.26	42
Above-ground biomass (g DM m <sup>-2</sup> )	27	0.91	25	24	0.78	19
Forage DM yield (g DM m <sup>-2</sup> )	33	0.75	21	24	0.86	27
N concentration (g kg <sup>-1</sup> DM)	33	0.46	16	24	-0.23	24
NDF (g kg <sup>-1</sup> DM)	33	-0.05	11	24	-0.35	12
dNDF (g kg <sup>-1</sup> NDF)	25	0.53	5	17	-0.81	8

Table 1. Model evaluation statistics of crop attributes of primary growth and regrowth in calibration and validation sets

N – number of measured/simulated data pairs; EF – model simulation efficiency; NRMSE – normalized root mean square error between simulated and measured values.

*Regrowth Nutrive Value*. The NRMSEs of all three attributes of nutritive value (N and NDF concentrations, and dNDF) of the regrowth were comparable to those of primary growth (Table 1) but the EF values were much lower, suggesting that the simulation of the regrowth nutritive value was not entirely successful and requires further research.

*Harvest Index*. The simulation of the harvest index was satisfactory, with EF greater than 0.38 and NRMSEs (6–13%) less than the range of coefficients of variation (13–30%) of measured data (Jing *et al.*, 2011). Simulation of the harvest index by using shoot apex height allows the estimation of residual biomass after harvest for different cutting heights.

#### Conclusions

New modules added to CATIMO successfully simulated the regrowth and the harvest index of timothy. Simulations of growth and nutritive value provide a framework to explore options for high yield and nutritive value of timothy.

#### References

Bélanger G., Tremblay G.F. and Mongrain D. (2008) Yield and nutritive value of the spring growth of an ageing timothy sward. *Canadian Journal of Plant Science* 88, 457–464.

Bonesmo H. and Bélanger G. (2002a) Timothy yield and nutritive value by the CATIMO model: I. Growth and nitrogen. *Agronomy Journal* 94, 337–345.

Bonesmo H. and Bélanger G. (2002b) Timothy yield and nutritive value by the CATIMO model: II. Digestibility and fiber. *Agronomy Journal* 94, 345–350.

Höglind M., Hanslin H.M. and Van Oijen M. (2005) Timothy regrowth, tillering and leaf area dynamics following spring harvest at two growth stages. *Field Crops Research* 93, 51–63.

Höglind M., Schapendonk A.H.C.M. and Oijen M.V. (2001) Timothy growth in Scandinavia: Combining quantitative information and simulation modelling. *New Phytologist* 151, 355–367.

Jing Q., Bélanger G., Baron V. and Bonesmo H. (2011) Modeling the biomass and harvest index dynamics of timothy. *Agronomy Journal* 103, 1397–1404.

Ruget F., Satger S., Volaire F. and Lelièvre F. (2009) Modeling tiller density, growth, and yield of mediterranean perennial grasslands with STICS. *Crop Science* 49, 2379–2385.

Thornley J.H.M. and Cannell M.G.R. (2000) Modelling the components of plant respiration: Representation and realism. *Annals of Botany* 85, 55–67.

Virkajärvi P., Pakarinen K., Hyrkäs M., Seppänen M. and Bélanger G. (2011) Tiller characteristics of timothy and tall fescue in relation to herbage mass accumulation. *Crop Science* 52, 970–980.

# Quality of hay and silage at Estonian riding stables

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## Abstract

The quality and nutritive value of locally produced silage and hay for horses were investigated. The forage comprised a mixture of grass crops. A total of 14 silage and 14 hay samples was collected from riding stables (housing 267 horses) and analysed from 2010–2011. Silage contained an average of 8.1 MJ kg  $DM^{-1}$  and hay 7.2 MJ kg  $DM^{-1}$  of metabolizable energy, and 56 g kg  $DM^{-1}$  and 22.5 g kg  $DM^{-1}$  digestible protein, respectively. The content of mycotoxins in silage was lower than in hay. The average zearalenon content of silage was 79.1 ppb whilst that of hay was 164.2 ppb. The deoxinivalenol content was 48.2 ppb in silage and 89.5 ppb in hay.

Keywords: hay, silage quality, mycotoxins, horses

## Introduction

Developments in silage baling technology have made it possible to prepare smaller portions of grass silage with the ideal composition for horses. Whereas hay is usually prepared from only the first cut of grassland, silage can be made from two cuts in the Nordic area. The third cut of grass has a very low DM yield, and therefore cannot be used as silage material for horses. Hay making has been the traditional conservation method of grass forage fed to horses. However, hay has to some extent been replaced by ensiled forage, as its preparation does not depend so much on weather conditions as hay production does, and high quality grass silage for feed can more easily be produced today than previously (Peiretti and Bergero, 2004). Therefore the use of silage for horses is becoming more widely accepted. The aim of this study was to determine the chemical composition, nutritive value and quality of both hay and silage used for feeding horses in Estonia.

## Materials and methods

A total of 14 silage and 14 hay samples were analysed during 2010–2011. The samples for analysis were collected from four riding stables that kept a total of 267 horses. The forage material comprised a mixture of grass crops. Additives were not used in the ensiling process. The samples were dried for 20 hours at 60°C, chopped to particles of 1 mm in diameter, and analysed for DM, crude protein, crude ash and crude fibre (AOAC, 2005). For determining the crude ash concentration, samples were ashed in a Phoenix–microwave furnace at 550°C. Crude protein was analysed by the Kjeldahl method with a Kjeltec 2300 analyser (FOSS Tecator Technology). The samples were analysed for crude fibre by the Fibretec System, and crude fat was determined with the Soxtec 2043 systems (FOSS).

The pH value was measured with a pH meter (Hanna Instruments pH meter 210). Ammonia nitrogen content was determined using an adjusted Kjeltec 2300 (FOSS) analyser. The ethanol, lactic acid and volatile fatty acids contents were determined chromatographically using an Agilent Technologies 7890A GC system with a column packed with 80/120 Carbopack B-DA/4% carbowax 20 M (Faithfull, 2002). The mycotoxin content of feed was determined by using the ELISA method with the Ridascreen ® FAST test kits. The laboratory data were analysed statistically using SAS software. The effects of chemical composition of feedstuffs

were tested by means of orthogonal contrasts. To analyse the traits containing zero values, ranks of values were used; other traits were converted into their logarithmic values.

#### **Results and discussion**

The chemical composition of the hay and silage is presented in Table 1. The nutrient composition of the samples was highly variable. The mean silage DM content was 486 g kg FM<sup>-1</sup> and that of hay was 850 g kg FM<sup>-1</sup>. The nutrient content of silage was higher than that of hay (P < 0.001). The silage prepared for the horses contained 8.1 MJ kg DM<sup>-1</sup> and the hay 7.2 MJ kg DM<sup>-1</sup> of metabolizable energy, and 56 g kg DM<sup>-1</sup> and 22.5 56 g kg DM<sup>-1</sup> digestible protein, respectively. According to Muhonen (2008), silage for horses should be made from grass crops the crude protein content of which does not exceed 120 g kg DM<sup>-1</sup>. Our data indicated that the content of crude protein in silage was only 109 g kg DM<sup>-1</sup>. This is an indication that the grass for silage had been cut too late. Silage produced from the grass crop cut at the optimum botanical maturity stage showed a higher nutritive value (Kaldmäe *et al.*, 2002).

	Silage		Hay $n = 14$			
Parameters	n = 14					
	$\overline{x}$	S	$\overline{x}$	S		
Dry matter, g kg <sup>-1</sup>	486	9.4	850***	1.5		
In dry matter:						
crude protein, g kg <sup>-1</sup>	109	3.4	63***	2.1		
crude ash, g $kg^{-1}$	77	1.9	45**	0.1		
crude fibre, g kg <sup>-1</sup>	302	3.1	336*	3.6		
crude fat, g kg <sup>-1</sup>	29	0.2	20***	0.1		
N-free extractives, g kg <sup>-1</sup>	484	4.5	535***	3.1		
metabolizable energy, MJ kg <sup>-1</sup>	8.1	0.4	7.2***	0.1		
digestible protein, g kg <sup>-1</sup>	56.0	24.3	22.5***	14.7		
*** <i>P</i> < 0.001; ** <i>P</i> < 0.01; * <i>P</i> <	0.05.					

Table 1. Chemical composition and nutritive value of silage and hay for horses

Table 2. Fermentation	quality	of	grass	silage
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Baramatara	Silage	Target value		
Faranieters	n = 14	(Kung and Shaver, 2001)		
Dry matter, %	48.6	45–55		
In dry matter:				
ethanol, g kg <sup>-1</sup>	5.2	5		
acetic acid, g kg <sup>-1</sup>	7.7	5–20		
propionic acid, g kg <sup>-1</sup>	0	0		
butyric acid, g kg <sup>-1</sup>	0.2	0		
lactic acid, g kg <sup>-1</sup>	18.7	20-40		
рН	5.1	4.7-5.0		
Ammonia-N of total N,%	3.3	<12		

Fermentation characteristics, pH, ammonia nitrogen, organic acids and ethanol contents are given in Tables 2. In general, it is recommended that lactic acids should comprise 65% of the total VFA content, and the lactic acid:acetic acid ratio should not be less than 3:1 (Kung and Shaver, 2001). Our research data revealed a 70% lactic acid content and 2.4:1 lactic acid:acetic acid ratio, respectively. The parameters indicated a normal fermentation quality of silage compared with the target value.

The presented feed quality characteristics also includes the concentrations of mycotoxins. The concentration of mycotoxins (zearalenon and deoxinivalenol) in hay and silage are shown in Table 3. Fungi and their associated mycotoxins are present both in hay and silage at different amounts each year, depending on climate and growing conditions (Nedělnik and Moravcova, 2006). The zearalenon content of silage was on average 79.1 ppb and the deoxinivalenol content was 48.2 ppb. In hay these concentrations were 89.5 and 164.2 ppb, respectively. Silage contained less mycotoxins compared to hay. The longer wilting time of grass crops in the field increased the content of mycotoxins in silage (Kaldmäe *et al.*, 2011).

Mycotoxin	Silage	Hay		
No of samples	14	14		
Zearalenon, ppb				
$\overline{x}$	79.1	89.5		
Min	20.0	0.01		
Max	135.6	249.6		
Deoxinivalenol, ppb				
$\overline{x}$	48.2***	164.2		
Min	0.01	30.0		
Max	158.9	358.7		

Table 3. Content of mycotoxin of silage and hay for horses

#### Conclusions

The nutrient content of the sampled silages was higher than that of hay samples. Silage had lower contents of mycotoxins compared to hay. Optimum harvesting of silage makes it possible to cover the nutrient requirements of horses.

#### References

AOAC. (2005) *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th ed. Association of Official Analytical Chemists International, Gaithersburg, MD, USA.

Faithfull N.T. (2002) *Methods in Agricultural Chemical Analysis: a practical handbook*, CABI Publishing, UK, 266 pp.

Kaldmäe H., Kärt O. and Vadi M. (2002) Effect of the growth stage of grasses and legumes on nutritive value and digestibility of silage. In: *Conference Proceedings of the XIIIth International Silage Conference* September 11–13, 2002. Auchincruive, Scotland, pp. 122–123.

Kaldmäe H., Olt A. and Ots M. (2011) Effect of additive on content of mycotoxins of grass silage. *Proceedings of the II International Symposium on Forage Quality and Conservation*, 16–19 Nov. 2011, Sao Pedro, pp. 427–428.

Kung L. and Shaver R. (2001) Interpretation and use of silage fermentation analysis reports. *Focus on For-age* 3, 13, 1–7.

Muhonen S. (2008) *Metabolism and hindgut ecosystem in forage fed sedentary and athletic horses*. Doctoral Theses Swedish University of Agricultural Sciences, Uppsala, 54 pp.

Nedělnik J. and Moravcova H. (2006) Mycotoxins and forage crops. Problems of the occurrence of mycotoxins in animal feeds. In: *Conference Proceedings of 12th International Symposium on Forage Conservation*. Brno, Czech Republic, pp. 13–25.

Peiretti P.G. and Bergero D. (2004) Grass silage as feedstuff for horses. *Journal of Food, Agriculture and Environment* 2, 182–185.

## Selenium fertilisation of grassland: effect of frequency and methods of application

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## Abstract

An adequate Selenium (Se) content in the diet of dairy cattle is important to ensure satisfactory animal fertility and health. The Se content of grassland forage of some European countries is very low, which makes dietary Se-supplementation necessary. As an alternative, the Se content in forage can be increased by grassland fertilisation. Se-containing fertilisers (SeF) are already available on the market in granulated form of Na<sub>2</sub>SeO<sub>4</sub> and BaSeO<sub>4</sub>, usually combined with limestone. There is increasing interest in combining SeF with slurry, in order to reduce application costs, but its effect is not known. For this reason, four fertilisation treatments (slurry mixed with SeF, slurry applied after spreading SeF, SeF mixed with mineral fertiliser, control with untreated slurry) were tested in a two-year experiment in combination with two application frequencies (yearly, once in two years). No effect of the fertilisation treatments was observed on yield. Se content in the forage was equally increased by all fertilisation. A single application of SeF in two years resulted in a Se content above, or close to, the recommended dietary level of 100  $\mu$ g kg<sup>-1</sup> until the first cut of the second year.

Keywords: fertilisation, slurry, fertilisation frequency, selenium, forage

## Introduction

Selenium (Se) is an essential micronutrient for livestock. It is part of the enzyme glutathione peroxidase (GSH-Px) which catalyses detoxification of peroxides (Lorenz and Boehnke, 1999). An adequate Se content in the diet of dairy cattle is important to ensure satisfactory animal fertility and health. Plant uptake depends primarily on the quantity of soluble Se  $(SeO_3^{2-} \text{ and } SeO_4^{2+})$  in the soil, which varies widely on the earth's surface (Shand *et al.*, 1992). In South Tyrol, there is evidence of low Se content in forage, which was found to be often below the recommended dietary level of 100 µg kg<sup>-1</sup> DM (Plattner *et al.*, 2005). Se deficiency makes dietary Se supplementation necessary. As an alternative, Se content in forage can be increased by grassland fertilisation. Se-containing fertilisers (SeF) are already available on the market, mainly combined with limestone. There is also an increasing interest in combining SeF with slurry, in order to reduce application costs, but its effect on forage yield and Se content in forage is not known. Also the definition of the necessary application frequency is of great importance, in order to reduce fertilisation costs.

## Materials and methods

A field experiment was established at the experimental farm Mair am Hof (920 m a.s.l., Dietenheim/Bruneck, South Tyrol, Italy) and conducted for two years under intensive management (four cuts year<sup>-1</sup>). The effect of four fertilisation treatments in spring (1: SeF mixed with slurry, 2: SeF applied before slurry application, 3: SeF mixed with mineral fertiliser, 4: control with slurry alone) and two application frequencies (once in two years, every year) on Se content in forage and forage yield were investigated. The plots were laid out as a randomized complete block design with three replications. In treatments 1, 2 and 4, 22 m<sup>3</sup> ha<sup>-1</sup> of 2:1 water-diluted slurry

were applied. Mineral fertilisation (N, P and K) of treatment 3 was arranged to match nutrient amount supplied by slurry, which was analysed at the beginning of each growing season (46 g kg<sup>-1</sup> DM content, 1.9 g kg<sup>-1</sup> N, 0.34 g kg<sup>-1</sup> P and 0.32 g kg<sup>-1</sup> K in 2010; 48 g kg<sup>-1</sup> DM content,  $2.0 \text{ g kg}^{-1} \text{ N}$ , 0.34 g kg $^{-1}$  P and 0.29 g kg $^{-1}$  K in 2011). In treatments 1 to 3, 10 g ha $^{-1}$  of Se were applied, corresponding to 25 kg ha<sup>-1</sup> of granulated SeF. The SeF contained 840 g kg<sup>-1</sup> CaCO<sub>3</sub>, 50 g kg<sup>-1</sup> MgCO<sub>3</sub> and 0.4 g kg<sup>-1</sup> Se, which was made up of 0.352 g kg<sup>-1</sup> BaSeO<sub>4</sub> and 0.048 g kg<sup>-1</sup> Na,SeO<sub>4</sub>. Fertilisers were manually applied. In fertilisation-treatment 1, SeF was thoroughly stirred with slurry for five minutes using a drilling machine equipped with a propeller blade (Ø 16 cm) at about 1100 rpm and then immediately spread using 15-litre watering cans. After each cut (1st to 3rd), 22 m<sup>3</sup> ha<sup>-1</sup> of biogas slurry were applied with an injector-equipped Terra-Gator 2244 (AGCO Netherlands B.V., Grubbenvorst, NL) irrespective of the fertilisation treatment. At each harvest date, a 1.35 m wide strip was mown in the middle of the plot and the fresh biomass weighed with a field scale. Dry matter content was determined on a 500 g sub-sample dried at 60°C. Se content was determined according to the EPA-Method 3052 (U.S. Environmental Protection Agency, 1996) using an ICP-MS (Mod. 7700, Agilent, Santa Clara, USA). The effect of fertilisation treatment, application frequency, harvest date and their interactions on forage DM vield and Se content was tested by means of a mixed model, taking into account the harvest date as a repeated factor with the plots as subject of repeated measurements. The design effects (block and harvest date  $\times$  block) were included in the model as well. Data of method 4 were not included in the analysis and are presented in tables and figures for reference only. Se content data were log-transformed to fulfil the requirements for ANOVA. Multiple comparisons were performed by LSD. A probability of P < 0.05 was considered to be significant.

#### **Results and discussion**

As already reported by other authors (Gissel-Nielsen, 1984), Se fertilisation did not affect DM yield over the whole observation period (P = 0.927). Fertilisation treatments also did not affect the Se content in forage (P = 0.665) and seem therefore to be equivalent (Table 1). It appears not to be relevant whether SeF is applied separately or mixed with slurry, nor whether the other nutrients are supplied by slurry or mineral fertilisers. However, caution should be adopted in the practice by mixing them with slurry, as BaSeO<sub>4</sub> is slowly soluble and both BaSeO<sub>4</sub> and Na<sub>2</sub>SeO<sub>4</sub> in the tested SeF are coat layers applied to clay prills. An inadequate mixing of slurry with SeF or too long times between mixing and spreading may lead to uneven distribution of SeF within slurry and in turn to high punctual application rates. This has to be avoided because of the narrow range between deficiency and toxicity of Se.

Table 1. Cumulated forag	e DM	yield and m	ean Se coi	ntent in forage de	pending on	fertilisatio	on method
and application frequenc	у						
Application frequency		Yearly		Once i	n two years	5	
Fertilisation method	1	2	3	1	2	3	4

Application free	luency_	Yearly Once in two years						
Fertilisation m	ethod	1	2	3	1	2	3	4
DM yield	2010	11.73	10.81	11.57	10.32	10.80	10.83	10.97
(Mg ha <sup>-1</sup> year <sup>-1</sup> )	2011	8.48	7.19	8.11	7.52	8.36	7.80	8.30
Se content	2010	225.8	158.9	219.3	172.6	209.8	251.5	28.0
$(\mu g \ kg^{-1} \ DM)$	2011	227.7	179.5	262.4	65.8	83.5	59.1	19.9

Both harvest date and fertilisation frequency, as well as their interaction (P < 0.001), affected the Se content. During the first growing season, the Se content steadily decreased from the first to the third harvest date and rose again at the fourth one (Figure 1). In the second year, a similar pattern with a less pronounced increase at the last harvest date was

observed. This effect was apparent also in other investigations (Gissel-Nielsen, 1984; Shand *et al.*, 1992). Fertilisation frequency affected the Se content throughout 2011. At the first two harvest dates, it was about five times higher in the plots fertilised yearly than in the plots fertilised once in two years. This difference declined over time and was about a twofold at the last harvest. On average, there was still evidence of a fertilisation effect two years after application. However, Se content in plots fertilised once in two years was below the recommended Se content in forage at all harvest dates in 2011 (Figure 1). In contrast, the Se content of the yearly fertilised treatments reached at the first cut of 2011 a level near to the upper range of the recommended Se content in forage (500  $\mu$ g kg<sup>-1</sup> DM) according to GfE (2001).



Figure 1. Change over time of Se content in forage in control and fertilised plots. Geometric means  $\pm$  SE are shown. The grey dashed line is the recommended Se content in forage. Significant effects of the fertilisation frequency within harvest dates are shown by asterisks

#### Conclusions

SeF increase the Se content in forage, irrespective of being applied in combination with slurry or mineral fertilisers. However, further studies about mixing systems are necessary to ensure that the method is feasible in the practice. A prosecution of this study is desirable to gain information about the cumulative increase of the Se content in forage due to a yearly application.

#### References

GfE (2001) Empfehlung zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt am Main, D, 136 pp.

Gissel-Nielsen G. (1984) Improvement of selenium status of pasture crops. *Biological Trace Element Research* 6, 281–288.

Lorenz F. and Boehnke H.-J. (1999) Selenversorgung des Weideviehs mit selenhaltigen Mineraldüngern. In: Arbeitsgemeinschaft Grünland und Futterbau (ed.) *Beiträge der 43. Jahrestagung der AGGF*, 26.–28.8. *1999 in Bremen*, Wissenschaftlicher Fachverlag Dr. Fleck, Gießen, D, pp. 184–187.

Plattner P., Cassar A., Stimpfl A. and Cozzi G. (2005) Der Selengehalt im Grünfutter des ersten Aufwuchses in Südtirol (Italian). *Laimburg Journal* 2, 79–84.

Shand C., Coutts G., Duff E. and Atkinson D. (1992) Soil selenium treatments to ameliorate selenium deficiency in herbage. *Journal of the Science of Food and Agriculture* 59, 27–35.

U.S. Environmental Protection Agency (1996) *EPA-Method 3052*, *Microwave assisted acid digestion of siliceous and organically based matrices*, U.S. Government Printing Office, Washington, USA.

# Production abilities and forage quality of selected species of *Bromus* family in the first year after renovation

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## Abstract

In 2008 a trial was established at the Jevíčko site on Fluvisol (345 m above sea level, 7.4°C, long-term rainfall average 545 mm) by a method of quick renovation of permanent grassland with three *Bromus* species: *Bromus inermis* Leysser cv. Tabrom (smooth brome), *Bromus marginatus* Nees es Steud cv. Tacit (mountain brome), *Bromus sitchensis* Trin. in Bong cv. Tambor (Alaska brome). The trial was fertilized with N 180 kg ha<sup>-1</sup> in the form of ammonium nitrate with lime, 35 kg ha<sup>-1</sup> P and 100 kg ha<sup>-1</sup> K; four-cut utilization, first cut on 29 April, then 45 days between cuts. Dry matter production in the first utilization year 2009 reached 14.12 t ha<sup>-1</sup> for smooth brome, 17.67 t ha<sup>-1</sup> for mountain brome, and 16.86 t ha<sup>-1</sup> for Alaska brome. The quality of forage dry matter in 2009 was evaluated by NIR Systems 6500. The parameters measured were crude protein, fibre, NEL (net energy of lactation), NEF (net energy of fattening). The objective is to compare production abilities and forage quality of three *Bromus* species in the conditions of the Czech Republic.

Keywords: permanent grassland, renovation, *Bromus inermis*, *B. marginatus*, *B. sitchensis*, yield, forage quality

## Introduction

*Bromus marginatus* Nees ex Steud. (Mountain brome) is an erect, short-living (4–7 years) bunchgrass, up to 1 m high, without rhizomes, botanically fitting in the frame of *Bromus* family into the section of *Ceratochloa* (DC et Beauv.) Griseb. It originates from North America where it appears as a native grass at 300-4000 m elevation. (Pavlick, 1995). It was brought to the Czech Republic (CR) in the period between the world wars (Míka and Řehořek, 2003), and nowadays it can be found on sandy soils, on river banks; for example, in the surroundings of the town of Veselí-upon-Lužnice (Veselí nad Lužnicí). Collection of seeds allowed breeding of a material that was registered as the cv. Tacit in 1998.

*Bromus sitchensis* Trin. in Bong. (Sitka brome, Alaska brome) comes from the Pacific coast of North America and it is used from south-east Alaska (the name comes from Sitka Island) down to Washington State (Pavlick, 1995). In the CR it demonstrates higher cold resistance than *B. catharticus*, it suffers less from leaf diseases, but it has a lower forage quality (Mika and Řehořek, 2003). It is popular in France due to its medium earliness in terms of harvest distribution (Míka and Řehořek, 2003). *Bromus inermis* Leysser (Smooth brome, Hungarian brome), which belongs to a steppe group, is characterized by good cold-resistance, persistence over 5 years, and medium competiveness. The objective of the study reported in this paper is to compare production abilities and forage quality of three *Bromus* species in the conditions of the Czech Republic.

#### Materials and methods

The Bromus species trial was established at Jevíčko in 2008 on a Glevic Fluvisol with neutral soil reaction (pH<sub>KCI</sub> 6.7) by a method of quick renovation of permanent grassland after the first cut by application of herbicide Touchdown Quatro (glyphosate) at the rate of 8 l ha<sup>-1</sup>. Bromus species were sown at the rate of 8 million germinating caryopses per hectare. The trial was fertilized with N 180 kg ha-1 in the form of ammonium nitrate, with lime, applied in three doses each of 60 kg ha<sup>-1</sup> (in spring, after the first and second cuts), 35 kg ha<sup>-1</sup> P (superphosphate) and 100 kg ha<sup>-1</sup> K (potassium salt) in 2009. The study evaluates the first harvest year of three brome species: Bromus inermis Leysser cv. Tabrom, Bromus marginatus Nees es Steud cv. Tacit, and Bromus sitchensis Trin. in Bong cv. Tambor. The trial was managed in four-cut utilization; first cut at the stage of stem extension, then 45 days between cuts. The contribution evaluates dry matter (DM) production and forage quality. The quality of forage DM in 2009 was predicted with NIR Systems 6500 fitted with a spinning sample module, in reflectance range 1100–2500 nm, band width 2 nm, measured in small ring cups, duplicate samples scanned twice. The parameters measured were crude protein (CP), fibre (CF), NEL (net energy of lactation), NEF (net energy of fattening), using software WinISI II, ver 1.50. The acquired findings were evaluated with variance analyses, the differences between averages were tested by the Tukey comparison test.

#### **Results and discussion**

In 2009, vegetation development was two weeks earlier in comparison with the long-term average due to an extraordinary early spring as a result of above-average temperatures in April (+5°C) in the whole Czech Republic, and the first cut was carried out on 29 April 2009. Of the evaluated three Bromus species (Table 1) B. marginatus demonstrated the highest dry matter production (17.67 t DM ha<sup>-1</sup>), then B. sitchensis (16.86 t ha<sup>-1</sup>), whereas B. inermis (14.12 t.ha<sup>-1</sup>) reached highly significantly lower yield (P < 0.01). Bromus *inermis* showed the highest forage quality, especially in concentration of CP (187 g  $kg^{-1}$  DM), the lowest fibre concentration (261 g  $kg^{-1}$  DM) and the highest PDIN concentration (107 g kg<sup>-1</sup> DM) in comparison with *B. marginatus* (CP, CF, PDIN:168; 274; 96); B. sitchensis had the lowest forage quality (153; 280; 87). Concentration of PDIE was almost identical in all evaluated species and it ranged from 83.2-81.9 g kg<sup>-1</sup> DM. Concentrations of NEL and NEF in the forage were at average levels, despite four-cut utilization and it reached 5.06–5.22 MJ NEL kg<sup>-1</sup> DM, whereas it was the lowest in B. sitchensis and the highest in B. marginatus; similarly for NEV values, which ranged from 4.77–4.94 MJ NEF kg<sup>-1</sup> DM; differences among species are inconclusive (statistically non-significant). The acquired findings are in agreement with Mika et al. (2004), where monocultures of mountain brome and smooth brome, and their mixtures with legumes demonstrated very good production abilities, medium competitiveness and average forage quality. The trials demonstrated that a mixture of mountain brome with alfalfa (87.5+12.5% seed weight) was very successful in terms of DM yield, nutritional quality, and also persistence (Míka et al., 2004).

Although mountain brome and smooth bromegrass are grasses with  $C_3$ -type of photosynthesis, their association ability with other grasses or legumes is quite low. Grasses with  $C_3$ -photosynthesis usually tend to grow in multi-variety stands, whereas grasses with  $C_4$ -type of photosynthesis usually grow in pure stands (Jones and Lazenby, 1988).

	DM	Quality parameters						
Grass species	DM	СР	Fibre	PDIN	PDIE	NEL	NEF	
	t ha <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	MJ kg <sup>-1</sup>	MJ kg <sup>-1</sup>	
Bromus inermis Leysser.	14.12	188	261	107	83	5.19	4.91	
Bromus marginatus Nees es Steud.	17.67	168	274	96	83	5.22	4.94	
Bromus sitchensis Trin. in Bong	16.86	154	280	88	82	5.06	4.77	
LSD <sub>0.05</sub>	2.49	27	22	15	4	0.41	0.48	
LSD <sub>0.01</sub>	2.90	31	26	17	4	0.47	0.56	

Table 1. Dry matter production and forage quality of Bromus species in 2009

LSD = low significant difference.

#### Conclusions

Production abilities of *B. sitchensis* cv. Tambor in the first harvest year after renovation are conclusively higher than those of *B. inermis* cv. Tabrom and inconclusively lower than those of *B. marginatus* cv. Tacit. Forage quality of *B. sitchensis* cv. Tambor is only medium, even under a four-cut utilization and lower in all evaluated parameters in comparison with *B. inermis* and *B. marginatus*. The productivity and chemical composition of these brome species, and their known attributes, suggest they are suitable for drier conditions, and in drought years, either as fodder or for bioenergy production.

#### Acknowledgements

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#### References

Jones M.B. and Lazenby A. (1988) *The grass crop, Physiological basis of production*. Chapman & Hall. Ltd., 369 pp.

Pavlick L.E. (1995) *Bromus of North America*. Royal Brit. Columbia Museum. Victoria, Canada, 160 pp. Míka V. and Řehořek V. (2003) (*Bromus L. sensu lato in Central Europe*) – *in Czech*. 1<sup>st</sup> edition, VÚRV Prague, 151 pp.

Míka V., Kohoutek A., Smrž J., Nerušil P., Odstrčilová V. and Komárek P. (2004) Performance of grass mixtures with mountain brome (*Bromus marginatus* Nees ex Steud.) in Central European lowlands. *Plant Soil and Environment* 50(3), 101–107.

# Conservation characteristics of maize cob ensiled with the addition of *Lactobacillus plantarum* MTD-1, *L. plantarum* 30114 or *L. buchneri* 11A44

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Abstract

Added bacteria can improve silage preservation and aerobic stability by altering fermentation patterns during ensilage. The aim of this study was to investigate the effects of contrasting lactic acid bacteria on the fermentation profile, chemical composition and aerobic stability of maize cob (rachis and grain). Cobs were separated from three replicate blocks of maize plants and precision chopped. Samples were allocated to the following treatments: no additive (control), *L. plantarum* MTD-1 (LP1), *L. plantarum* 30114 (LP2) and *L. buchneri* (LB). Added bacteria were applied of  $1 \times 10^6$  colony forming units/g fresh herbage. Samples were ensiled in laboratory silos at 15°C for 130 days. The dry matter, neutral detergent fibre, starch and crude protein concentrations of cob after ensilage were unaffected (*P* > 0.05) by bacterial addition. The proportion of lactic acid in total fermentation products was higher (*P* < 0.05) for silages inoculated with LP2, compared to the control silages or LB silages. Silages treated with LB were more aerobically stable (*P* < 0.01) than silages made with LP1 or LP2.

Keywords: maize, cob, bacteria, silage

## Introduction

The use of bacterial additives, selected to dominate the epiphytic bacteria on herbage and alter the silage fermentation process, can reduce conservation losses and improve nutritive value. Those which promote highly efficient lactic acid dominated fermentations, such as *Lactobacillus plantarum*, have the potential to reduce fermentation losses, whereas lactic acid bacteria (LAB) that promote the production of anti-fungal compounds such as acetic acid or (e.g. *L. buchneri*) during ensilage can increase the aerobic stability of maize silage and reduce aerobic losses. Forage maize (*Zea mays* L.) plants consist of two physical components with contrasting chemical compositions: the starch-rich cob (grain and rachis) and the fibre-rich stover (stem, leaves, husks and tassel). However, information on the effects of added bacterial on the ensilage of the cob component of maize produced in marginal growing areas, such as regions of Ireland, is not readily available. The aim of this study was to investigate the effects of inoculating maize cob with three different LAB on the fermentation profile, chemical composition and aerobic stability of the subsequent silages.

## Materials and methods

Cob was separated from whole-crop maize plants obtained from three replicate field blocks. Sub-samples were precision-chopped and allocated to one of the following treatments: no additive (control), *Lactobacillus plantarum* MTD-1 (LP1), *L. plantarum* 30114 (LP2) and *L. buchneri* 11A44 (LB). Each bacterial additive was applied of 1×10<sup>6</sup> colony forming units/g fresh herbage. Triplicate samples of each treatment were ensiled in laboratory silos at 15°C for each of 3, 10, 35 or 130 days. The fermentation products (lactic acid, acetic
acid, propionic acid, butyric acid and ethanol) after each ensiling duration and the chemical composition, dry matter (DM) recovery and aerobic stability after 130 days ensilage were measured as described by McEniry *et al.* (2006). In addition, LAB plate counts and real-time quantitative PCR (qPCR) were carried out at each time-point to estimate *L. plantarum* and *L. buchneri* population sizes based on the method described by Schmidt *et al.* (2008). Data to assess the nutritive value, DM recovery and aerobic stability and deterioration (i.e. day-130 only) in response to additive treatments were subjected to a 1-way analysis of variance, whereas all other data were subjected to a 2-way analysis of variance for a 4 (additive treatment)  $\times$  4 (ensilage duration) factorial arrangement of treatments using the PROC GLM procedure of the SAS statistical program. Treatment contrasts were made using the Fisher least significant differences test.

#### **Results and discussion**

The DM, starch, neutral detergent fibre and crude protein concentrations of cob after 130 days ensilage were unaffected (P > 0.05) by additive treatment. Inoculation of cobs with LB, LP1 or LP2 did not affect (P > 0.05) silage DM, lactic acid, acetic acid, ethanol, propionic acid, butyric acid, or total fermentation product (TFP) concentrations at any stage during ensilage, compared to control silages (Table 1).

Table 1. Fermentation products and microbial enumerations of cob silages made	with different bacte-
rial additives for 4 ensilage durations	

Stage of ensiling		3 d	ays			10 0	lays			35 0	lays			130	days		s.e.m. <sup>12</sup>		Sig	3
Additive <sup>1</sup>	С	LB	LP1	LP2		Α	Е	AxE												
DM <sup>2</sup>	456	449	451	445	463	443	453	451	452	447	444	432	446	452	455	466	6.6			
pH	3.8	4.1	3.9	3.9	3.9	3.9	3.8	3.7	3.8	4.0	3.8	3.7	3.9	4.1	3.8	3.7	0.10	**		
Lactic acid3	12	12	11	16	13	13	10	11	16	14	11	17	12	7	16	6	3.4			
Acetic acid3	2.0	2.0	1.3	1.8	2.0	1.7	1.1	1.3	2.1	3.8	1.6	2.6	4.9	7.6	2.7	5.3	0.76	*	***	
Ethanol <sup>3</sup>	1.1	1.4	1.2	1.1	2.7	2.2	1.8	1.8	3.1	2.9	4.0	2.9	5.3	5.4	5.0	3.7	0.46		***	
Propionic acid3	0.2	0.1	0.2	0.2	0.3	0.2	0.1	0.1	0.2	0.2	0.1	0.8	0.4	0.7	0.3	2.1	0.42			
Butyric acid3	0.0	0.1	0.1	0.0	0.2	0.1	0.0	0.0	0.1	1.3	0.1	0.6	0.4	1.3	0.2	1.8	0.44		*	
TFP <sup>3</sup>	15	16	13	19	18	17	13	15	21	22	16	22	23	22	25	19	3.9			
NH <sub>3</sub> -N <sup>4</sup>													84	115	92	95	10.9			
LA/TFP <sup>5</sup>	0.76	0.70	0.79	0.81	0.72	0.70	0.76	0.71	0.68	0.62	0.61	0.71	0.50	0.32	0.65	0.38	0.094		***	
AA/TFP <sup>6</sup>	0.15	0.18	0.10	0.11	0.11	0.12	0.08	0.12	0.12	0.18	0.12	0.10	0.23	0.35	0.12	0.25	0.043	*	***	
LAB (plate count) <sup>7</sup>	8.21	8.68	8.81	8.96	8.02	8.69	8.28	8.42	8.02	8.50	7.55	7.00	7.92	7.81	7.45	6.48	0.321	*	***	
L. buchneri (qPCR) <sup>8</sup>	3.65	6.33	3.86	3.70	4.12	8.40	4.10	3.15	6.11	6.88	4.95	4.19	7.56	7.64	5.74	5.35	0.513	***	***	*
L. plantarum (qPCR) <sup>8</sup>	4.35	5.20	7.70	5.95	4.95	7.00	6.99	5.61	6.15	5.99	7.76	6.29	6.37	6.18	6.44	5.51	0.336	***	*	**
DM recovery9													940	916	930	968	17.0			
$Hours > 2^{\circ}C^{\scriptscriptstyle 10}$													123	192	75	97	17.8	*		
ACT 120h11													6.0	0.1	13.7	9.2	2.72	*		

<sup>1</sup>C = uninoculated control, LB = *Lactobacillus buchneri* 11A44, LP1 = *L. plantarum* MTD-1, LP2 = *L. plantarum* 30114;

<sup>2</sup> g kg<sup>-1</sup>; DM = dry matter;

<sup>3</sup> g kg<sup>-1</sup> DM; TFP = total fermentation products (Lactic acid + acetic acid + propionic acid + butyric acid + ethanol)

<sup>4</sup> g kg<sup>-1</sup> N; Only analysed after 130 days ensilage;

<sup>5</sup> g lactic acid per g TFP

<sup>6</sup> g acetic acid per g TFP

<sup>7</sup>  $Log_{10}$  (colony forming units using agar per g herbage); LAB = lactic acid bacteria

<sup>8</sup> Log<sub>10</sub>(estimated colony forming units using real time quantitative PCR per g herbage); L = Lactobacillus<sup>9</sup>g silage per kg herbage ensiled (dry matter basis); Only determined after 130 days ensilage <sup>10</sup> Interval (h) until temperature rises more than 2°C above reference temperature (index of aerobic stability); Only determined after 130 days ensilage

<sup>11</sup> Accumulated temperature rise during 120 hours exposure to air (index of aerobic deterioration); Only determined after 130 days ensilage

<sup>12</sup> Standard error of the mean

<sup>13</sup> A = additive, E = ensiling stage; \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001.

The qPCR data revealed that *L. buchneri* populations were more abundant (P < 0.001) in control silages than in silages made using LP1 and LP2. Although *L. buchneri* populations were higher (P < 0.01) after 35 days ensilage in silages prepared using LB, no difference (P > 0.05) was observed between control and LB silages ensiled for the other ensilage durations. Therefore, the lack of effect of the addition of LP1 or LP2 on the silage fermentation of cob, compared to the control, was likely due to a failure of these organisms to dominate the epiphytic populations of *L. buchneri*. Cobs treated with LP1 had a lower (P > 0.05) acetic acid proportion in TFP than when treated with LP2, indicating contrasting effects of these different strains of the same species on the silage fermentation. The recovery of ensiled DM in cob silages was unaffected (P > 0.05) by additive treatment. Cob silages made with LB and ensiled for 130 days were more (P < 0.05) aerobically stable and underwent less (P < 0.05) acrobic deterioration than silages made with LP1. This reflected the higher (P < 0.05) concentrations of acetic acid in LB compared to LP1 silages. Acetic acid inhibits yeast growth and thus, helps reduce aerobic deterioration (Muck, 2010).

#### Conclusions

The aerobic stability and DM recovery of cob silages in this study were not improved when made with *L. buchneri* 11A44, *L. plantarum* MTD-1 or *L. plantarum* 30114, due to the indigenous highly heterolactic fermentation that prevailed in the uninoculated cob during 130 days ensilage.

#### References

McEniry J., O'Kiely P., Clipson N.J.W., Forristal P.D. and Doyle E.M. (2006) The microbiological and chemical composition of baled and precision-chop silages on a sample of farms in County Meath. *Irish Journal of Agricultural and Food Research* 45, 73–83.

Muck R.E. (2010) Silage microbiology and its control through additives. *Revista Brasileira de Zootecnia* 39, 183–191.

Schmidt R.J., Emara M.G. and Kung Jr. L. (2008) The use of a quantitative real-time polymerase chain reaction assay for identification and enumeration of *Lactobacillus buchneri* in silage. *Journal of Applied Microbiology* 105, 920–929.

# Postponing of the first harvest in semi-natural grasslands: decline in nutrient concentrations?

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# Abstract

Postponing of the first harvest until summer is widely incorporated into agri-environmental schemes. In agriculturally improved grasslands a late harvest usually provides forage with much lower N, P and K concentrations than an early harvest. The question is how postponing of first harvest affects nutrient concentrations in biomass if late harvest is applied in semi-natural grasslands for the first time. A rapid seasonal decline of N, P and K concentrations in biomass was recorded in formerly unmanaged *Bromion* but not in formerly grazed *Cynosurion* and *Violion* grasslands. Approximately 50% of the grassland biomass consisted of species with persistent leaves in *Cynosurion* and *Violion*, while the proportion of these species was very low in *Bromion*. Total amounts of N, P and K in standing biomass were substantially higher in *Bromion* than in the other two grasslands in the spring, whereas no differences between sites were observed in the summer. Nutrient dilution and resorption in *Bromion* versus long leaf lifespan strategy in *Cynosurion* and *Violion* explain the different seasonal patterns of N, P and K concentrations. In conclusion, a one-time application of a late harvest need not decrease N, P and K concentrations in forage from grasslands formerly grazed in early spring.

Keywords: agri-environmental schemes, grazing, late harvest, leaf persistence, nutrient resorption

#### Introduction

The most frequent agri-environmental measure in grasslands is postponing of the first harvest until summer in order to assure reproduction of plants, insects and ground-nesting birds. In agriculturally improved grasslands a late harvest provides forage with much lower N, P and K concentrations than an early harvest. However, such sharp decline in nutrient concentrations is not necessarily recorded in unfertilized semi-natural grasslands (Donath *et al.*, 2004). In infertile environments plants have to conserve nutrients (Eckstein *et al.*, 1999), which is ensured either by nutrient resorption (supporting seasonal decline in nutrient concentrations at approximately the same level for the whole season). The main aim of this study was to test whether postponing of the first harvest until summer decreases nutrient concentrations in forage less in grasslands with a higher proportion of species with persistent leaves.

#### Materials and methods

Three unfertilized grasslands (White Carpathian Mts, Czech Republic) were selected: (1) *Bromion* (49°05'58" N, 18°01'59" E), unmanaged 13 years before the start of the study, formerly managed by cattle grazing. (2) *Cynosurion* (48°56'20" N, 17°48'00" E), rotationally grazed by cattle from early spring for more than 25 years prior to the start of the study.

(3) Violion (48°53'47" N, 17°34'44" E), an old, eroded and continuously grazed pasture on a hil-Itop with more than 50 years history of sheep grazing beginning each year in early spring. Within each grassland we randomly selected 15 plots (each 1 m<sup>2</sup> in size) for biomass sampling in spring and another 15 plots in summer. In 2004 the spring standing biomass was sampled on 19 May in Bromion and Violion and on 7 June in Cynosurion (due to delayed growth at a higher altitude) and on 30 June at all sites for the summer term. Plots were neither grazed nor cut before sampling thus providing first growth biomass on all sampling dates. Prior to clipping, the biomass proportions of species in a sample were estimated. A total of 80 species was recorded from the three sites at both sampling dates. The leaf persistence was extracted from the BIOLFLOR database (Klotz et al., 2002): 46 species possessed leaves green only in the vegetation period and 34 species had persistent evergreen leaves (often living more than one year). Community leaf persistence was calculated using a binary coded variable (1 for species with persistent leaves) by weighting the trait values of species with their proportions in the biomass sample, thus community leaf persistence of a sample could range from 0 to 1. The biomass was harvested 3 cm above ground, dried at 55°C and taken to the laboratory for analyses of nutrient concentrations.

#### **Results and discussion**

The most productive *Bromion* exhibited a steep decrease in concentrations of N, P and K from spring to summer. In contrast, *Cynosurion* and *Violion* were characterized by relatively unchanged concentrations of N and P while concentrations of K increased (Figure 1). These results are in line with Alonso and García-Olalla (1997). Comparison of concentrations with the levels recommended for dairy cattle showed that the biomass was poor in P at all sites. With the exception of spring harvests in *Bromion* and *Violion*, N concentrations were below or at the lower limit of dairy cattle requirements. K concentrations higher than dairy cattle requirements are usually observed in many types of grasslands. However, the nutrient concentrations satisfy the lower nutritional requirements for beef cattle and sheep.

A gradient in community leaf persistence was recorded in the order *Bromion < Cynosurion < Violion* (Table 1). Total amounts of N, P and K in standing biomass in the spring were highest in *Bromion* that contained a relatively low proportion of species with persistent leaves (29% of biomass). In contrast, the lowest spring amounts of N, P and K were found in *Violion* with 64% of biomass created by species with persistent leaves. The total amounts of nutrients harvested in summer were similar in all grasslands and corresponded to the average values found by Smits *et al.* (2008) for peak standing biomass in unfertilized *Bromion erecti* grassland (50, 4 and 60 kg ha<sup>-1</sup> of N, P and K, respectively). These divergent seasonal patterns match the differences in the ecophysiology of the dominant species: *Brachypodium pinnatum* in *Bromion* and *Festuca rubra* in both *Cynosurion* and *Violion*. It is known that *B. pinnatum* shows rapid senescence and retranslocation of nutrients into underground rhizomes, and that *F. rubra* typically keeps the leaf nutrient concentrations at the same level throughout the season.

Table 1. Average community leaf persistence and total amounts of nutrients (kg ha<sup>-1</sup>) of harvested aboveground biomass from all sites in spring and summer. Two-way ANOVA, cells with the same letter row-wise are not significantly different at P = 0.05 (Tukey HSD test)

Site	Broi	nion	Cyne	osurion	Violion		
Date	spring	summer	spring	summer	spring	summer	
Leaf persistence	0.29 <sup>ab</sup>	0.14 <sup>a</sup>	0.45 <sup>cd</sup>	0.41 <sup>bc</sup>	0.64 <sup>e</sup>	0.58 <sup>de</sup>	
Total N	40.2 <sup>bc</sup>	55.9 <sup>d</sup>	30.5 <sup>b</sup>	39.3 <sup>bc</sup>	16.5ª	51.9 <sup>cd</sup>	
Total P	3.7 <sup>b</sup>	4.2 <sup>b</sup>	2.9 <sup>b</sup>	3.6 <sup>b</sup>	1.2ª	4.2 <sup>b</sup>	
Total K	44.2 <sup>bc</sup>	69.4 <sup>d</sup>	41.0 <sup>b</sup>	55.8 <sup>bcd</sup>	13.1ª	59.6 <sup>cd</sup>	



Figure 1. Dry matter standing biomass and concentrations of nutrients. Two-way ANOVA, error lines represent SE, bars with the same letter are not significantly different at P = 0.05 (Tukey HSD test). Punctuated lines indicate typical concentrations of nutrients in *Lo-lium perenne* (standard forage grass) in optimum time for harvest; grey zones indicate concentrations of nutrients recommended for nutrition of dairy cows (Whitehead, 2000)

#### Conclusions

Postponing first harvest until summer does not necessarily decrease N, P and K concentrations in forage from grasslands, where abiotic conditions and the long-term management regime support a high proportion of species with persistent leaves.

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#### References

Alonso I. and García-Olalla C. (1997) Mineral composition of soils and vegetation from six mountain grassland communities in northern Spain. *Journal of the Science of Food and Agriculture* 73, 200–206.

Donath T.W., Hölzel N., Bissels S. and Otte A. (2004) Perspectives for incorporating biomass from nonintensively managed temperate flood-meadows into farming systems. *Agriculture, Ecosystems and Environment* 104, 439–451.

Eckstein R.L., Karlsson P.S. and Weih M. (1999) Leaf lifespan and nutrient resorption as determinants of plant nutrient conservation in temperate-arctic regions. *New Phytologist* 143, 177–189.

Klotz S., Kühn I. and Durka W. (eds) (2002) *BIOLFLOR – Eine Datenbank zu biologisch-ökologischen Merkmalen der Gefäßpflanzen in Deutschland, Schriftenreihe für Vegetationskunde 38.* Bundesamt für Naturschutz, Bonn, Deutschland.

Smits N.A.C., Willems J.H. and Bobbink R. (2008) Long-term after-effects of fertilization on the restoration of calcareous grasslands. *Applied Vegetation Science* 11, 279–286.

Whitehead D.C. (2000) Nutrient elements in grassland, soil-plant-animal relationships. CABI Publishing, Wallingford, UK, 369 pp.

# Evolution of forage quality of selected grass species during the first harvest regrowth

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#### Abstract

This contribution evaluates dry matter increase and changes in concentrations of nutrients and energy of 7 grass species and varieties from 7 sequential samplings at weekly intervals from the end of April to mid-June 2011. The trial was established in 2010 at the Jevíčko site on glevic fluvisol (345 m above sea level, 7.4°C mean annual temperature, long-date rainfall average 545 mm) with seven grass species (Dactylis glomerata L. cv. Vega, Festuca arundinacea L. cv. Kora, Festulolium cv. Hykor and cv. Hostyn, Festuca pratensis L. cv. Kolumbus, Lolium perenne L. cv. Kentaur (4n) and Bromus inermis Leysser cv. Tabrom). The trial was fertilized in 2011 with N at 120 kg ha<sup>-1</sup> as ammonium nitrate with lime, applied in two doses of 60 kg ha<sup>-1</sup> (in spring, and after the first cut) plus 35 kg ha<sup>-1</sup> P and 100 kg ha<sup>-1</sup> K. The 7 sampling dates during first-cut growth were from 28 April and at weekly intervals until mid-June. The average dry matter (DM) production at first harvest was 2.20 t ha<sup>-1</sup> (range 1.60–2.76 t ha<sup>-1</sup> DM). The average yield increase reached 0.96 t DM ha<sup>-1</sup> and within species it ranged from 0.80-1.09 t DM ha<sup>-1</sup>. The concentration of crude protein (CP) in forage at first harvest was 181.8 g kg<sup>-1</sup> DM; CF 176.3 g kg<sup>-1</sup> DM, NEL 6.10 MJ kg<sup>-1</sup> DM, and NEF 6.08 MJ kg<sup>-1</sup> DM. During the accrual period the CP concentration decreased by 16.6 g kg<sup>-1</sup> DM (11.7–20.2), the fibre increased by 23.7 g kg<sup>-1</sup> DM (21.3–26.9), NEL concentration decreased by 0.23 MJ kg<sup>-1</sup> DM (0.18–0.27), NEF concentration by 0.29 MJ kg<sup>-1</sup> DM (0.23–0.34) for each week of growth.

Keywords: renovation, forage quality, time of harvest, *Dactylis glomerata*, *Festuca arundinacea*, Festulolium, *Festuca pratensis*, *Lolium perenne*, *Bromus inermis* 

#### Introduction

The recent increase of milk production per cow in dairy cattle in the Czech Republic (over 7000 kg FCM) presents increased demands on fodder quality. The quality of forage changes a lot during the first harvest regrowth. Individual species within mixtures of grasses and legumes show significant differences in earliness (1–3 weeks), and even greater differences in forage quality (3–4 weeks) (Pozdíšek *et al.*, 2002). In order to determine an optimum date for harvest for each individual species, sequential samplings were analysed during the first harvest regrowth.

#### Materials and methods

A trial was established in 2010 at the Jevíčko site on gleyic fluvisol (345 m above sea level, 7.4°C mean annual temperature, long-date rainfall average 545 mm) by a method of quick renovation with seven grass species/cultivars (*Dactylis glomerata* L. cv. Vega (DG), *Festuca arundinacea* L. cv. Kora (FA), Festulolium cv. Hykor (Fl-Hy) and cv. Hostyn (Fl-Ho), *Festuca pratensis* L. cv. Kolumbus (FP), *Lolium perenne* L. cv. Kentaur (4n) (LP) and *Bromus inermis* Leysser cv. Tabrom (BI)). Grasses were sown in a randomised block design in pure-stands with four replications. The trial was fertilized in 2011 with 120 kg N ha<sup>-1</sup> as ammonium nitrate with lime, applied in two doses of 60 kg ha<sup>-1</sup> (in spring,

and after the first cut), and 35 kg ha<sup>-1</sup> P (superphosphate) and 100 kg ha<sup>-1</sup> K (potassium salt) were applied. There were seven sampling dates during the first growth, with the first cut on 28 April 2011 and subsequent samplings at weekly intervals until mid-June. The quality of forage dry matter (DM) in 2011 was evaluated by NIR Systems 6500 fitted with a spinning sample module, in reflectance range 1100–2500 nm, band width 2 nm, measured in small ring cups, with duplicate samples being scanned twice. The measured parameters were crude protein (CP), fibre (CF), NEL (net energy of lactation), NEF (net energy of fattening), using software WinISI II, vers. 1.50 with the aim to determine yields, nutrient content and energy concentration changes during the first harvest growth. Characteristics were statistically evaluated using an analysis of variance and a method of linear regression; correlation coefficients were calculated and critical values  $r_{\alpha}$  (n = 7) of correlation coefficient for  $\alpha = 0.05$  and  $\alpha = 0.01$  evaluated.

#### **Results and discussion**

The year 2011 was characterized by a quick arrival of spring, with temperatures above average (by +2.6°C), and below-average rainfall (–16 mm) in April and early May, which clearly accelerated vegetation development by 1 to 2 weeks. The average DM production of the studied species at the first sampling date (28 April) was 2.20 t ha<sup>-1</sup>, with the highest DM production for Fl-Ho (2.76 t ha<sup>-1</sup> DM) and the lowest for BI (1.60 t ha<sup>-1</sup>). The average weekly DM growth rate, evaluated with linear regression (Table 1a) reached 0.96 t DM ha<sup>-1</sup> and ranged within the evaluated species from 0.80 (FP) to 1.09 (Fl-Ho) t DM ha<sup>-1</sup>. The differences between species are statistically high significant (P < 0.01; Table 1b).

Table 1a. Parameters of linear equation y = a+bx characterizing the evolution of dry matter production and concentration of CP, CF (g kg<sup>-1</sup>) net energy of lactation (NEL) and net energy of feeding (NEF) (MJ kg<sup>-1</sup> DM) related to time, in the period of first harvest accrual in 2011 from sequential grass sampling (n = 7) – the first sampling in the last week of April

								Pa	rame	ter						
		DM	(t ha=		CD	- 11	DM		Fibre			NEL (	MJ	N	VEF (1	MJ
Species	Cultivar	DM	(t na	· DM)	CP (	g kg ·	DM)	(g	kg <sup>-1</sup> I	DM)		kg <sup>-1</sup> D	M)	k	g-1 DI	M)
1		R.	.p.		R.	p.		R.	р.		R	p.		R	.p.	
		а	b	- 1	а	b	1	а	b	- 1	А	b	1	а	b	1
DG	Vega	0.88	0.98	0.95**	196.2	-18.8	0.94**	145.6	22.3	0.99**	6.57	-0.27	0.99**	6.67	-0.33	0.99**
FA	Kora	1.54	0.91	0.95**	196.1	-18.0	0.94**	151.9	22.6	0.98**	6.63	-0.22	0.98**	6.37	-0.28	0.98**
Fl-Hy	Hykor	1.56	0.86	0.96**	175.2	-15.6	0.93**	162.7	21.3	0.96**	6.13	-0.18	0.97**	6.13	-0.23	0.97**
Fl-Ho	Hostyn	1.65	1.09	0.98**	169.2	-14.0	0.90**	134.1	24.7	0.97**	6.38	-0.22	0.96**	6.47	-0.28	0.96**
FP	Kolum- bus	1.27	0.80	0.96**	195.1	-18.1	0.92**	127.5	26.5	0.98**	6.57	-0.27	0.98**	6.66	-0.33	0.98**
LP	Kentaur	0.58	1.08	0.98**	162.0	-11.7	0.92**	118.4	21.8	0.96**	6.40	-0.19	0.98**	6.51	-0.24	0.97**
BI	Tabrom	0.50	1.00	0.98**	219.8	-20.2	0.93**	132.5	26.9	0.99**	6.53	-0.27	0.99**	6.62	-0.34	0.99**
Species	average	1.14	0.96	0.98**	187.7	-16.6	0.94**	139.0	23.7	0.98**	6.42	-0.23	0.99**	6.49	-0.29	0.99**
Table 11	o. Analys	sis of	vari	ance												
Source																
of vari-	df	SS	F <sub>test</sub>	Sig.	SS	F <sub>test</sub>	Sig.	SS	F <sub>test</sub>	Sig.	SS	F <sub>test</sub>	Sig.	SS	F <sub>test</sub>	Sig.
ability																
A (spe-	6	32	11	**	138	21	**	273	28	**	1	5	**	2	7	**
cies)	0	52	11		156	21		215	20		1	5		2	/	
B (cuts)	6	720	247	**	2311	259	**	4478	464	**	39	252	**	60	263	**
Total	108	923			2860			5143			46			72		
D						1.4.	a a affi		10-	1	~ f f			~~~~~	af an	

R.p. = regression parameters, r = correlation coefficient, df = degree of freedom, SS = sum of squares,  $F_{test}$  = Fisher test, Sig. = statistically significant ( $P_{0.05}$  = \*;  $P_{0.01}$  = \*\*).

The average CP content of forage at the first sampling was 181.8 g kg<sup>-1</sup> DM, CF content was 176.3 g kg<sup>-1</sup> DM, NEL content was 6.10 MJ kg<sup>-1</sup> DM, and NEF content was 6.08 MJ kg<sup>-1</sup> DM. During accrual period the concentration of CP decreased by 16.6 g kg<sup>-1</sup> DM (within the range 11.7 [in LP] to 20.2 [in BI]); the fibre increased by 23.7 g kg<sup>-1</sup> DM (within the range 21.3 [in Fl-Hy] to 26.9 [in BI]); NEL concentration decreased by 0.23 MJ kg<sup>-1</sup> DM (within the range 0.18 [in Fl-Hy] to 0.27 [in BI]), which was slightly less than the value of 0.26 MJ kg<sup>-1</sup> DM observed by Pozdíšek *et al.* (2002) in grass forage; NEF concentration decreased by 0.29 MJ kg<sup>-1</sup> DM per week (within the range 0.23 [in Fl-Hy] to 0.34 [in BI]). Correlations with time of all equations of linear regression are highly conclusive (P < 0.01). Low NEL concentration in *Lolium perenne* L. was caused in 2011 by a considerable drought at the beginning of the vegetation period; this species with its shallow root system has difficulties with nutrient uptake, and drought reduces its growth rate and yield (Garwood and Sinclair, 1979; Thomas, 1986) unlike *Dactylis glomerata, Festuca arundinacea* and Festuloliums, which tolerated better drought, thanks to their deeper root systems.

#### Conclusions

Prospective grass species for highly productive dairy cows are Fl-Hy 'Hostyn' and FP 'Kolumbus', harvested in mid-May. Suitable grass species for suckler cows are FA 'Kora' and BI 'Tabrom' (BI), harvested in the second half of May.

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#### References

Garwood E.A., and Sinclair J. (1979) Use of water by six grass species. 2. Root distribution and use of soil water. *Journal of Agricultural Science, Cambridge* 93, 25–35.

Pozdíšek J., Míka V., Kohoutek A., Jakešová H. and Němcová-Schuplerová P. (2002) Nutritive value of selected grass species (cultivars) and tetraploid red clover in sequential sampling. *Grassland Science in Europe* 7, 150–151.

Thomas H. (1986) Water use characteristics of *Dactylis glomerata* L., *Lolium perenne* L. and *Lolium multiflorum* Lam. plants. *Annals of Botany* 57, 211–223.

# Optimising the application technique for silage additive in harvesting machinery

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### Abstract

Trials showed that the current practice of spraying additive (a mix of formic acid and ammonium formate) only on the top of the ingoing forage in loader wagons results in uneven distribution of additive in the forage, compared with the evenness achieved in precision choppers. This means a quality risk for silage made with loader wagons. When half of the dose of additive was sprayed on the ingoing forage from below, and half from above, the evenness of distribution was improved. In the best case it became as good as in the precision chopper. For the application from above, narrow jets from a perforated pipe caused less loss of acid-based additive through evaporation and wind drift than the wide fans from flat-fan nozzles. In a towed chopper, the best place of application was the lower part of the chute. In a self-propelled chopper, in which the forage stream is thicker, the additive was better mixed with the forage when applied before the knife cylinder so that its mixing effect was utilised.

Keywords: silage additive, application technique, evenness, loss, loader wagons, precision choppers

#### Introduction

Additives (preservatives) generally improve silage quality. For a good result, the additive has to be distributed evenly and dosed with the correct quantity in the forage. The objective of this study was to identify the best application methods in loader wagons and in towed (tractor-driven) and self-propelled precision choppers, regarding evenness and losses of application. Losses typically arise from evaporation and wind drift of additive droplets. In this text, 'loader wagon' means a self-loading wagon with a rotor that pushes the herbage through a row of *stationary* knives (rather than a self-loading wagon with an integrated precision chopper with *rotating* knives).

#### Materials and methods

In trial 1, the application methods in Table 1 were compared in a loader wagon and a towed precision chopper. In trial 2, the application methods in Table 2 were compared in the same machines as in trial 1. Methods C and D were included in both trials. In trial 3, the methods in Table 3 were compared in a self-propelled precision chopper. Methods A and B (Table 1) represent the current practice in loader wagons: additive is sprayed on the windrow in front of the pickup, or on the forage flow on the pickup. C is a new method that we developed with the aim to get more even distribution: half of the additive dose was sprayed from above on the pickup, and half underneath the grass flow on the pickup. For this, a plastic pipe with 20 mm outer diameter and 1.3 mm holes at an interval of 100 mm was fastened to the pickup surface, so that the grass slid over it. In both A, B, C and D, the application from above was done through flat-fan nozzles. In methods 4 and 5, the perforated plastic pipe had holes with 1.3 mm diameter at an interval of 60 mm. The pipe was perpendicular to the direction of the grass flow in the inlet channel between the pickup and the knife rotor. In method 5, the normally open top side of the inlet channel was covered with a plastic film. In the self-propelled chopper (Table 3), the first method was a plastic pipe with 1.5 mm holes at an interval of 33 mm placed above the

front opening of the inlet channel. Injection in the curved chute was on the outer (grass) side/ arc or the inner (air) side/arc. In trials 1 and 2, four loads per method were tested, and eight grass samples per load analysed for formic acid. In trial 3, five loads per method were tested, and ten samples per load analysed for formic acid. The evenness of application was calculated as the loadwise coefficients of variation (CV) for the formic acid concentration of the samples from the load. The mean CV for each application method was calculated, and the differences in CV between the methods were statistically tested with analysis of variance. The loss of additive was determined as the difference between the amount of additive consumed from the additive vessel per load and the amount of additive in the grass of each load, calculated from the load weight and the formic acid concentration in the samples. In trial 1 the additive was AIV Prima (620 g kg<sup>-1</sup> formic acid, 240 g kg<sup>-1</sup> ammonium formate). In trials 2–3 the additive was AIV 2 Plus (760 g kg<sup>-1</sup> formic acid, 55 g kg<sup>-1</sup> ammonium formate). Wind speed and temperature in trial 1: 0-2.5 m s<sup>-1</sup>, 15-25°C, trial 2: 0-6.0 m s<sup>-1</sup>, 12-21°C, trial 3: 0-0.6 m s<sup>-1</sup>, 15-21°C. The trials had a randomised complete block design. The statistical analyses were based on the following mixed model:  $y_{ii} = \mu + \tau_i + \rho_i + \varepsilon_{ii}$  where  $\mu$  is the overall mean,  $\tau_i$  is the fixed effect of the application method,  $\rho_i$  is the random effect of block and  $\epsilon_{ii}$  is the random error term of the model.

#### **Results and discussion**

The new method C in the loader wagon distributed the additive with significantly better evenness (CV 50%) than the traditional methods (CV 79-84%, Table 1). In trial 2, however, both varieties of the new method (C and 9, Table 2) resulted in significantly worse evenness (CV 89%) than the precision chopper (CV 23-46%). This does not necessarily contradict the result from the first trial that the new method seems better in evenness than the traditional ones in loader wagons, because the second trial did not include any such comparison. It is logical that the current practice in loader wagons, to spray the additive only on top of the ingoing forage, results in uneven distribution. The additive remains on the surface of the windrow or forage flow. The feeding rotor has a limited mixing effect and does not even out the uneven distribution. Precision choppers have a fast rotating knife cylinder which mixes forage and additive. The new method improves the preconditions to get good silage quality with loader wagons. In trial 2, replacing flat-fans (C) with narrow jets from a perforated pipe (9) reduced the losses from 47 to 28%. Water is less sensitive to evaporation than acid. Therefore the reduction of loss might be smaller with biological additives in water solution than with acid-based additives. In the towed chopper, methods 1, D, 3 and 4 are not worth recommending because they involve a risk of bigger losses in the open space in windier conditions. Methods 5 and 6 can be considered the best ones when taking both evenness and loss into account. Method 6 can be recommended in practice, since it is easier to install than 5 where the user himself has to make the perforated pipe. It is logical that method 7 causes uneven distribution, the additive does not get enough of travel distance to mix with the forage when it is added to the forage stream in the last moment before leaving the machine.

In the self-propelled chopper, the distribution was significantly better with application at the inlet channel than in the chute (Table 3). This is logical: application in the inlet channel utilises the mixing effect of the knife rotor and the accelerator. In the chute, the stream of herbage is thick, and additive applied at the surface of the thick stream is not mixed but remains at the surface of the stream. However, application in front of the feeding channel brought acid odour into the cabin, which is unpleasant for the driver. This could probably be avoided with application further back in the channel, but still before the accelerator to ensure good mixing. Application in the chute resulted in good evenness in the towed chopper but not in the self-propelled one. The reason is probably that the forage stream in the chute of self-propelled choppers is thicker and

denser than in towed ones, so the injected additive penetrates the forage stream worse in the chute of self-propelled choppers. Acidic additives corrode machinery to some extent, which can make some users unwilling to apply them in the inlet channel. Application in the top deflector saves the machine from corrosion. However, fodder quality should be considered more important than corrosion. The new method in loader wagons requires more fitting work from the user than current equipment, and would therefore be promoted if the manufacturers/importers would make and install the perforated pipes.

Table 1. Evenness and loss of additive in loader wagon and towed precision chopper, trial 1. (Treatments with the same letter in the CV were not significantly different)

Machina	Application method	Evenness	Loss	D
wiachine	Application method	CV, %	%	1
	A. From above, in front of pickup	79.3 <sup>A</sup>	48.3	
Loader wagon	B. From above, at pickup	83.6 <sup>A</sup>	33.9	A versus B:
	C. From above + jets under, at pickup	49.7 <sup>в</sup>	32.9	0.01 <p<0.05< td=""></p<0.05<>
Towed precision chopper	D. From above in open inlet channel	46.2 <sup>B</sup>	42.0	

CV = coefficient of variation (standard deviation divided by mean). The smaller CV, the better evenness.

Table 2. Trial 2. The percentage given for flat-fan nozzles is the ratio between the real flow (l/min) and their nominal flow at 1 bar (nominal size). The smaller ratio, the coarser spray. The statistical significance of differences between methods was calculated for methods 5, 7, C and 9 versus the other methods, and for C versus 9. \*  $0.05 \ge P \ge 0.01$ , \*\* $0.01 \ge P \ge 0.001$ , \*\*\*  $P \le 0.001$ . The methods which are most recommendable in each machine, for reasons explained in the text, are bolded

Machin	ePlace of application	Nozzle and spray type	Even p	ness of ap- lication	Loss		
		(n) = number of nozzles abreadth	CV, %	b P	Loss,%	Р	
	1. Pickup	flat-fan nozzles, coarse spray 33% (5)	) 27.9		16.9		
-	D. Open inlet channel	flat-fan nozzles, fine spray 150% (5)	31.4		22.4		
Towed	3. Open inlet channel	flat-fan nozzles, coarse spray 29% (3)	45.5		7.55		
prec.	4. Open inlet channel	perforated pipe; solid jets	29.3		13.5		
chonne	r 5. Covered inlet channel	perforated pipe; solid jets	22.8	* vs. 3, 7	9.22		
enoppe	6. Chute, lower part	solid-jet nozzles (3)	26.0		6.78		
	7. Top deflector	solid-jet nozzles (2)	45.5		1.19	*vs. D	
	C. Flat fans from above	+ solid jets under, at pickup	89.5	*** vs. 1,	47.2	***vs. 1, D,	
				D, 3, 4, 5,		3, 4, 5, 6, 7	
Loader				6, 7, 9			
wagon	9. Solid jets both above	and under, at pickup	89.0	*** vs. 1,	28.2	*vs. C.	
				D, 3, 4, 5,		**vs. 3, 5, 6.	
				6, 7, 9		***vs. 7	

Table 3. Evenness of additive in a self-propelled chopper, and percentage of grass which got too little additive

Location of Application	Evenn	ess of application	% of the herba less additi	ge which got ive than	Applied
	CV, %	Significance A–B	$1.51 t^{-1}$	3.0 1 t <sup>-1</sup>	dose I t
Front of inlet channel	20 <sup>A</sup>	-	0	0	5.1
Chute base, outer (grass) side	61 <sup>B</sup>	**P = 0.0017	2	22	5.8
Chute base, inner (air) side	49 <sup>в</sup>	* P = 0.0154	10	24	4.4
Chute, top deflector	64 в	***P = 0.0009	14	36	4.9

#### Conclusions

The current practice of spraying additive only on top of the ingoing forage in loader wagons results in uneven distribution of additive in the forage. Spraying half of the additive on the forage from below improves the evenness of distribution, and reduces the risk of uneven silage quality. For the application from above, narrow jets from a perforated pipe cause less loss of acid-based additive through evaporation and wind drift than flat-fan nozzles. Smaller losses of additive can be achieved in precision choppers than in loader wagons. In towed precision choppers, the best place of application is the lower part of the chute. In self-propelled choppers, the best place is in the inlet channel before the accelerator.

# Influence of NIRS-method on the calibration of N-, ash- and NDF-content of grassland hay and silage

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# Abstract

Near Infrared Spectroscopy (NIRS) is a common method to analyse the quality of grassland biomass. However, the effort required for sample preparation and measurement could restrict the sampling rate and prediction quality. To assess suitable approaches for bioenergetic recovery of grassland biomass, we developed NIRS-calibrations for N-, ash- and NDF- content of hay and silage of botanically very diverse grassland communities. Six different NIRS-methods of increasing sample preparation and standardization of measurement conditions were applied and compared.

The coefficient of determination ( $r^2$ ) and the ratio of prediction to deviation (RSC) were defined as quality parameters for method comparison. Assuming a good calibration model at RSC  $\ge 2$  and  $r^2 \ge 0.9$  all methodological approaches on hay samples allowed high calibration accuracy for N, which increased with increasing method standardization. Calibrations of ash and NDF content were good on methods carried out with laboratory near infrared systems, but on field spectrometric systems they were only adequate (RSC < 2). Calibrations on silage samples did not deliver appropriate calibration quality.

Keywords: NIRS, bioenergy, hay, silage, grassland

#### Introduction

Requirement for efficient process control in bioenergy recovery of biomass is an early quality assessment. Near infrared spectroscopic methods are established for the quantification of van Soest detergent components and structural carbohydrates, which are connected to bioenergetic parameters such as methane yield and higher heating value. However, extensive pre-measurement sample preparation sets limits in sampling intervals and it delays information acquisition. Thus, it is of lower value for online process control. Whilst NIR reflection signals rely heavily on sample geometry and environmental conditions during measurement, calibration accuracy does not necessarily increase with increasing sample preparation and measurement standardization. For example, Terhoeven-Urselmanns *et al.* (2007) developed better calibrations for fresh samples of Chinese white cabbage than for dried samples. As there is no comparison available for high diversity grassland communities, we developed six NIRS-methods with increasing sample preparation and measurement standardizations of nitrogen-, ash- and NDF content of hay and silage samples of highly diverse grassland communities were used to compare and evaluate the applicability of the methods in the context of bioenergy recovery.

#### Materials and methods

This study was carried out alongside the Jena Experiment (Roscher *et al.*, 2004). The experimental design covers a high diversity gradient of 1 to 60 species of typical European *Ar*-*rhenateretum* communities. Aboveground biomass of the 82 plots for NIRS measurements and chemical analysis was harvested 5 cm above the soil surface in the  $3\times3$  m core area of

each plot twice in the years 2008 and 2009. Subsamples for hay measurements were dried for 72h at 65°C. Subsamples for silage measurements were ensiled without silage additives in 2l glass vessels. Spectra were collected in six methodological approaches differing in sample preparation and measurement standardization.

Distance fieldspectroscopy on hay samples (DFH):

Spectra for the least standardized method for hay samples was conducted with a field spectrometry system (Fieldspec 3, ASDInc., USA) at a distance of 67 cm above the samples in a lightproof hardware device. Samples were cut to a maximum length of 10 cm and stored in a topless nonreflecting box. The measurable sample surface covers a total area of 0.07 m<sup>2</sup> and was constantly illuminated by three 50W tungsten halogen bulbs. The spectral device was calibrated according to the manufacturer's specification with a white calibration panel right before each measurement. Each sample was measured three times and was mixed between measurements to reduce bulk effects caused by litter dispersion.

Contact field-spectroscopy on hay samples (CFH):

The lightproof hardware device was replaced by the Fieldspec3 Plant-Probe-Foreoptic accessory to carry out measurements in direct contact with the sample. The accessory design covers a sampling area of 0.5 cm<sup>2</sup>. Twenty measurements were executed, distributed equally throughout the box.

Coarse samples laboratory spectroscopy on hay (CLH):

The third approach was carried out on a Foss XDS Rapid-Content-Analyzer laboratory NIR system (Hillerød, Denmark) with the large area coarse sample cell (40 cm<sup>2</sup> measuring surface). Samples were measured twice after being milled with a Retsch cutting mill (SM 100, Haan, Germany, sieve diameter: 6 mm).

Ground samples laboratory spectroscopy on hay (GLH):

The method representing the highest sample treatment was performed on the XDS with a circular quartz cuvette. Hay samples were ground to a maximum grain size of 1 mm with a FOSS sample mill (CyclotecTM 1093, Haan, Germany).

Silage spectra were recorded in two ways using the ASD field spectrometer (Method DFS) and the FOSS XDS NIRS system (Method CLS). Measurements were conducted analogous to method DFH and method CLH using ensiled samples without further treatment.

Reference values for nitrogen were determined with an elemental analyzer (Vario Max, CHN). Approximations for sample ash content were defined by combustion in a muffle furnace at 550°C for 12 h.

Reference values for NDF content were determined by Van Soest detergent fibre method and near infrared spectroscopy. Calibration development was performed for every method on averaged spectra of each sample using WinISI III (version 1.63) calibration software package (Infrasoft International, Port Matilda, Pensylvania USA). Spectral outliers were removed in two elimination steps before calibration was performed with a modified partial least square regression (MPLS).

#### **Results and discussion**

The coefficient of determination  $(r^2)$  and the ratio of standard deviation of reference values to standard error of cross validation (RSC) were defined evaluation parameters for method

comparison. According to Chang *et al.* (2001) a calibration model is considered good at  $RSC \ge 2$  and  $r^2 \ge 0.9$  and becomes unreliable at RSC < 1.4 and  $r^2 < 0.8$ .

All methods on hay samples allowed a high calibration quality for N (RSC > 2 and  $r^2$  between 0.82 and 0.98), which increased along method standardization (DFH<CFH<CLH<GLH) with the most remarkable gap between the methods on the laboratory systems GLH and CLH (Table 1). Calibrations of ash- and NDF content were good in GLH and CLH but decreased to being of only adequate calibration quality in DFH (RSC < 2;  $r^2 < 0.8$ ). With RSC and  $r^2$  values of 1.4 and below 0.8, respectively, calibrations for methods applied on silage samples (CLS and DFS) did not reach sufficient prediction accuracy according to our criteria and would only be suitable for an initial screening (Schimleck *et al.*, 2003).

Method	N (%	DM)	Ash (%	6 DM)	NDF (% DM)		
Wiethou -	RSC	r <sup>2</sup>	RSC	$r^2$	RSC	r <sup>2</sup>	
GLH	5.58	0.98	3.07	0.93	3.42	0.92	
CLH	2.53	0.87	2.73	0.90	2.58	0.87	
CFH	2.18	0.82	1.83	0.80	2.08	0.80	
DFH	2.40	0.88	1.68	0.74	1.90	0.75	
CLS	1.69	0.73	1.42	0.55	1.68	0.67	
DFS	1 40	0.73	1 27	0.47	1 45	0.70	

Table 1. Quality parameters for applied methods on calibration of N-, ash- and NDF-content. RSC: ratio of standard deviation of reference values to standard error of cross validation;  $r^2$ : determination coefficient of calibration

#### Conclusions

Results from this study suggest that all NIRS methods applied on hay samples are adequate for prediction of nitrogen content. Therefore, nitrogen content of high diversity grassland communities can be predicted with low sample pretreatment. On the other hand, ash and NDF content should be analysed with laboratory spectrometric methods to obtain reliable results. Silage samples were not reliably predictable by either of the applied methods.

#### References

Chang C.W., Laird D.A., Mausbach M.J. and Hurburgh Jr. C.R. (2001) Nearinfrared reflectance spectroscopy-principal components regression analyses of soil properties. *Soil Science Society of America Journal* 65, 480–490.

Roscher C., Schumacher J., Baade J., Wilcke W., Gleixner G., Weisser W.W., Schmid B. and Schulze E.-D. (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology* 5, 107–121.

Schimleck L.R., Doran J.C. and Rimbawanto A. (2003) Near infrared spectroscopy for cost-effective screening of foliar oil characteristics in a *Melaleuca cajuputi* breeding population. *Journal of Agricultural and Food Chemistry* 51, 2433–2437.

Terhoeven-Urselmans T., Bruns C., Schmilewski G. and Ludwig B. (2007) Quality assessment of growing media with near-infrared spectroscopy: chemical characteristics and plant assays. *European Journal of Horticultural Science* 73(1), 28–36.

# Automation and control system of tractor and loader wagon in forage harvesting

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### Abstract

An intelligent control system for a tractor-loader wagon combination was developed for controlling the driving speed of the machine and the dosing of silage additive. The speed was automatically adjusted to keep the mass flow constant, thus decreasing the risk of blockage in the wagon. The electronic control unit estimates mass flow of forage using a Kalman filter. The filter estimates mass flow based on three measurements: volume flow of swath calculated from a laser scanner in the front of the tractor, load mass measured with pressure sensors in the wagon, and density of swath derived from a NIR (near infrared) sensor in the front wall of the wagon. The speed controller is based on fuzzy logic, regulating the speed on the basis of the tractor's driving speed and engine RPM, estimated mass flow, and changes in the swath cross-sectional area. The additive is applied to forage according to the estimated mass flow. When the target mass flow was 30 kg s<sup>-1</sup>, the measured mass flow mean was 29.1 kg s<sup>-1</sup> with a standard deviation of 4.15 kg s<sup>-1</sup>. The NIR moisture readings deviated between -6.17 and 5.49% of the oven-dried samples.

Keywords: forage harvesting, loader wagon, automation, fuzzy logic, intelligent

#### Introduction

The agricultural industry has striven to improve functions and features of their products by researching the human-machine interface. Control and automation can increase the efficiency of agricultural machines, which is a strong selling feature. For example, forage harvesting with a tractor and loader wagon often requires many observations and simultaneous corrections or adjustments by the driver. The ISO11783 (a.k.a. ISOBUS) standard for communication between tractors and agricultural implements provides a common platform for implementing new control systems and accelerates their adoption.

Loader wagons have increased in size and capacity, and have become fast and efficient harvesting machines. Maintaining the optimal load of loader-wagon feeding unit can prevent problems such as overloading, blockages, or unevenly chopped grass from underloading. The chop length is an important factor, which can affect silage quality and the performance of feeding equipment (Suokannas and Nysand, 2008). The use of the precise amount of additive applied at the harvesting phase requires continuous monitoring by the driver. This paper presents a solution for optimizing the forage harvesting process with an intelligent control system developed for controlling the driving speed of the machine combination and the dosing of silage additive. In this study the test tractor had an ISO11783 class 3 TECU (Tractor electronic control unit) and an ISO11783 bus was fitted to the loader wagon.

#### Materials and methods

The research platform was based on an evaluation version of a Valtra T132 tractor equipped with a continuous power transmission and a Krone ZX 45-GL loader wagon equipped with

hydraulic suspension. The swath cross-sectional area was measured with a laser scanner in front of the tractor and the moisture of the forage with a NIR (near infra-red) sensor fitted to the front wall of the wagon. The weight of the load was measured with three pressure sensors, two in the rear axle's hydraulic circuits (160 bar) and a third at the front of the wagon (250 bar). The position of the pick-up unit of the wagon was monitored with a sensor, and the speed of the scraper floor was measured with a pulse sensor. The additive pump was equipped with its own ECU (electronic control unit) and a PID (Proportional-Integral-Derivative), which is the most widely used feedback controller. The additive was applied through four nozzles situated in the fixed boom above the pick-up. The loader wagon ECU estimated mass flow of forage using a Kalman filter based on three inputs: volume flow of swath, total mass of collected forage in the wagon and the density of forage in the swath. All measurements were delivered via an ISOBUS installed in loader wagon.

The weighing systems were calibrated with concrete weights varying from 1000 to 8000 kg and a NIR–sensor with oven-dried grass samples varying from 15 to 50% DM. All data were collated with a Labview-program.

The harvesting trials were done mainly in two fields: a uniform 5 ha field in second cut of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward, and a 2 ha field in second cut of red clover (*Trifolium pratense*). The forage was mown with a 3.2 m mower conditioner. After wilting for 3 to 24 hours, it was windrowed in widths of 9 to 12 m.

#### **Results and discussion**

When the area of the swath decreased, the speed of the tractor was increased (Figure 1) and the target of 30 kg s<sup>-1</sup> constant mass flow was achieved with relatively low variation in the measured mass flow; average measured mass flow was 29.1 kg s<sup>-1</sup>.



Figure 1. Optimized speed control based on mass flow of grass. Notice the scale of the swath area is multiplied by 10 to distinguish it from the X-axis

The control system kept the mass flow of grass in the feeding unit constant and optimal regardless of the swath area and mass, thus preventing blockages, improving work efficiency and quality of grass chop. The control of additive application prevents too low or too high consumption. The optimized process is easier to operate (Figure 2).

The main problem to overcome was the measurement system for forage mass in the wagon using pressure sensors due to retardation and acceleration forces impacting on the wagon and friction in the hydraulic cylinders.



Figure 2. Measured flow of additive compared with the target flow applied to grass

The NIR moisture readings deviated between -6.17 and 5.49% from the oven-dried samples. Research by Thurner *et al.* (2011) found the absolute deviation for the DM content in online measurement in self-propelled forage harvester for system A (NIR sensor) of between -0.97 and -6.81% and for system B (dielectric conductivity and temperature of the crop) between +0.46 and -6.57% when compared with the reference values.

#### Conclusions

There are several different kinds of yield measurement and control systems in forage harvesting machines, but no others use a forage mass estimator and apply a fuzzy logic speed controller and precision additive application. The speed controller was able to maintain the mass flow at a desired level and apply additive with an accurate ratio.

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#### References

Suokannas A. and Nysand M. (2008) Loader wagon compared to metered chopper for forage harvest. *Grassland Science in Europe* 13, 648–650.

Thurner S., Fröhner A., Köhler B. and Demmel M. (2011) Online measurement of yield and dry matter of wilted grass with two forage harvesters – comparison with and vertification of reference measurements. In: Stafford J.V. (ed.), *Precision agriculture 2011*. The 8<sup>th</sup> European Conference on Precision Agriculture, Prague, Czech Republic 11–14 July 2011, pp. 628–637.

# Effect of mineral nitrogen fertilization and water-deficiency stress on chemical composition of lucerne (*Medicago sativa* L.)

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### Abstract

To study the effect of mineral nitrogen (N) fertilization and water-deficiency stress on chemical composition of lucerne (*Medicago sativa* L.) grown for forage, a pot trial was carried out at the Institute of Forage Crops, Bulgaria. Effects of mineral fertilization at the doses of 40, 80, 120 and 160 mg N kg<sup>-1</sup> soil were examined. Ten-days water-deficiency stress was achieved at the stage of budding of lucerne by interrupting the irrigation until soil moisture was reduced to 37–40% of Field Capacity. It was found that with increasing doses of mineral N fertilization to 160 mg N kg<sup>-1</sup> soil, the crude protein content in dry aboveground mass increased. When 160 mg N kg<sup>-1</sup> were applied, production, relative to that of the unfertilized control, was increased by up to 11% under optimal moisture, and by up to 8% for water-deficiency stress conditions. Ca:P ratio in above-ground dry mass remained almost unchanged in the conditions of optimal moisture, and increased significantly in the conditions of water-deficiency stress by 24–29%.

Keywords: mineral nitrogen fertilization, water deficiency stress, chemical composition, lucerne

#### Introduction

The question of whether nitrogen-fixing crops such as lucerne require some nitrogen (N) fertilization, or can rely solely on that obtained by nitrogen fixation, is debatable in the literature (Oliveira *et al.*, 2004; Werner and Newton, 2005; Tufenkci *et al.*, 2006). This debate is of greater importance under conditions of water-deficiency stress (Vasileva *et al.*, 2006). The aim of this work was to study the effect of mineral N fertilization and water-deficiency stress on chemical composition of lucerne grown for forage.

#### Materials and methods

A pot experiment using the lucerne variety Victoria was carried out at the Institute of Forage Crops, Pleven, Bulgaria (2003–2004). Pots of 10 L capacity and soil subtype of leached chernozem were used. Sowing was conducted by hand at a depth of 2–3 cm with a about 20 germinable seeds per pot. After emergence, four well-developed plants per pot were allowed to remain. The following treatments were tested with four replications: Under optimum water supply – (75–80% of Field Capacity) (FC): 1. Control 1- unfertilized- N0PK (C1); 2. Soil + 40 mg N kg<sup>-1</sup> soil (N40PK); 3. Soil + 80 mg N kg<sup>-1</sup> soil (N80PK); 4. Soil + 120 mg N kg<sup>-1</sup> soil (N120PK); 5. Soil + 160 mg N kg<sup>-1</sup> soil (N160PK). Under 10-day water deficiency stress (37–40% FC): 6. Control 2- unfertilized- N0PK (C2); 7. Soil + 40 mg N kg<sup>-1</sup> soil (N40PK); 8. Soil + 80 mg N kg<sup>-1</sup> soil (N80PK); 9. Soil + 120 mg N kg<sup>-1</sup> soil (N120PK); 10. Soil + 160 mg N kg<sup>-1</sup> soil (N160PK). For treatments 1 to 5, 75–80% of FC was maintained by daily watering, and for 6 to 10, water-deficiency stress was achieved at the stage of budding of lucerne by interrupting the irrigation until soil moisture was reduced to 37–40% of FC. Mineral nitrogen (N) as ammonium nitrate equivalent to the tested doses was applied. All treatments were treated against a background of P and K, the phosphorus

being applied as triple super phosphate, and potassium as KCl (P – 110 mg P kg<sup>-1</sup> soil; K – 110 mg K kg<sup>-1</sup> soil). Two harvests were undertaken. Aboveground and root mass were dried at 60°C. Crude protein content (CP) was determined according to Kjeldahl method (CP = N × 6.25), crude fibre (CF) by Weende method, phosphorus (P) by hydroquinon, and calcium (Ca) – complexometrically (AOAC, 1990). The data from two experimental years were statistically processed using SPSS 10.0.

#### **Results and discussions**

Mineral nitrogen fertilization is one of the main factors influencing the chemical composition of plants. CP and CF content are important quality characteristics for legumes. The data in our study (Table 1) show, that with increasing doses of N fertilization, CP content in aboveground dry mass increased under optimal moisture conditions (in agreement with Tufenkci *et al.*, 2006), as well as under water-deficiency stress. When 160 mg N kg<sup>-1</sup> were applied to soil there was increased production compared to the unfertilized control, by up to 11% for optimal-moisture conditions, and up to 8% for water-deficiency stress. At the same dose of N fertilization the CP content decreased by 10–11% under both conditions of water supply.

	Dry above	e-ground mass	Dry roo	ot mass
Treatments	СР	CF	СР	CF
		g kg <sup>-1</sup>	DM	
		optimal moisture	e (75–80% FC)	
N0PK (C1)	167.4	305.5	115.5	232.0
N40 PK	166.2	308.2	107.9	231.8
N80 PK	170.3	298.8	110.8	235.5
N120 PK	172.5	302.9	124.0	233.0
N160 PK	185.9	276.4	132.3	216.5
SE (P = 0.05)	3.5	5.7	4.5	3.4
		water deficiency str	ess (37-40% FC)	
N0PK (C2)	158.5	321.6	120.0	231.3
N40 PK	154.0	311.6	126.5	235.7
N80 PK	165.6	296.4	115.1	221.6
N120 PK	171.8	295.9	138.7	211.5
N160 PK	171.4	286.8	137.7	199.9
SE (P = 0.05)	3.5	6.2	4.7	6.5

Table 1. Crude protein and crude fibre content in dry aboveground and dry root mass of lucerne after mineral nitrogen fertilization

At the lower doses tested (40 and 80 mg N kg<sup>-1</sup> soil) and under optimal moisture, the CP and CF content in dry root mass decreased by 7 and 4%, respectively, compared to unfertilized control. For the doses of 120 and 160 mg N kg<sup>-1</sup> soil, CF increased by 7–15% for optimal moisture, and by 15–16% for water-deficiency stress. The higher CP content in dry root mass was a protective reaction of the plants to the stress factor.

Water-deficiency stress had a stronger depressive effect on CF content in dry root mass than in the dry above-ground mass.

In our study the Ca:P ratio in above-ground dry mass remained almost unchanged for optimal moisture conditions, except at 160 mg N kg<sup>-1</sup> soil (Figure 1). The changes in Ca content due to N fertilization were probably related to the participation of this element in the processes of N assimilation (Follet and Wilkinson, 1995). Due to the higher Ca content in the conditions of water-deficiency stress, the Ca:P ratio increased significantly.

The Ca:P ratio in dry root mass varied considerably under both water-supply conditions.



Figure 1. Ca:P ratio in dry above-ground and dry root mass of lucerr after mineral nitrogen fertilization

#### Conclusions

Increasing doses of mineral nitrogen fertilization applied to lucerne increased the crude protein content in the above-ground dry mass. When 160 mg N kg<sup>-1</sup> were applied to the soil, production was increased, relative to that of the unfertilized control, by up to 11% for optimal moisture conditions, and by up to 8% under water-deficiency stress.

#### References

AOAC (1990) Official methods of analysis of the Association of Official Analytical Chemists. 15th ed., Association of Official Analytical Chemists, Arlington VA, USA.

Follet R.F. and Wilkinson S.R. (1995) Nutrient management of forages. In: Barnes R.F., Miller D.A. and Nelson C.J. (eds) *Forages* Vol. II. The Science of Agriculture, 5<sup>th</sup> edn. Iowa State University Press, Ames, Iowa, pp. 55–82.

Oliveira W., Oliveira P., Corsi M., Duarte F. and Tsai S. (2004) Alfalfa yield and quality as function of nitrogen fertilization and symbiosis with *Sinorhizobium meliloti*. *Sci. Agric*. (Piracicaba, Braz.) 61, 433–438. Tufenkci S., Erman M. and Sonmez F. (2006) Effect of nitrogen application and *Rhizobium* inoculation on the yield and nutrient uptake in lucerne (*Medicago sativa*). *Bulletin of Pure and Applied Sciences-Botany* 25, 1. Vasileva V., Kostov O. and Vasilev E. (2006) Development of lucerne (*Medicago sativa* L.) treated with mineral fertilizer and manure at optimal and water deficit conditions. *Comm. Appl. Biol. Sci. Ghent University* 71, 5–17.

Werner D. and Newton W.E. (2005) Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment. Springer.

# Chemical composition dynamics of alfalfa (*Medicago sativa* L.) at different plant growth stages

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### Abstract

Alfalfa (*Medicago sativa* L.) is a valuable forage species because of its high yield and nutritional value. The production and quality are influenced by the management, soil and pedoclimatic conditions. An essential factor of alfalfa forage quality is the plant growth stage at harvesting. Research was conducted in the forest steppe of NE Romania  $(47^{\circ}05'-47^{\circ}10' \text{ N} \text{ and } 27^{\circ}28'-27^{\circ}33' \text{ E})$ . We recorded the influence of fertilization and plant growth stage at harvest on production of dry matter (DM) per hectare, the leaves/stems ratio and on components of feed quality. The results showed that fertilization influenced positively the DM production, with variations of the leaves/stems ratio, whereas the plant growth stage at harvest influenced the feed chemical composition

Keywords: fertilization, plant growth stage, quality, Romania

# Introduction

Alfalfa (*Medicago sativa* L.) is one of the most valuable forage plants due to its high production and feed quality. The quantity-quality relationship is very important and is influenced by climatic conditions and management. Among the elements of management of alfalfa cultivation, fertilization and harvest time are two important links that affect its production level and quality. Mineral or organic fertilization improves the productivity of alfalfa, especially under soil conditions with low content of nutritive elements (Delgado *et al.*, 2001; Oliveira *et al.*; 2004). Stage of development at harvest determines the feed value. The more the harvest time is delayed, the more the plants' content in CP decreases while NDF and ADF increase (Lamb *et al.*, 2003; Tyrolová and Výborná, 2008; Rimi *et al.*, 2010). The leaves / stems ratio is an important quality indicator and is influenced by the time of harvest. Leaves contain twice the amount of CP as the stems, which is why it is desirable to have a high percentage of leaves in the harvest (Lamb *et al.*, 2007; Petkova and Panayotov, 2007).

#### Materials and methods

The research was performed in 2011, at the Research Station of the University of Agricultural Sciences and Veterinary Medicine Iasi in Romania. The soil is a cambic chernozem, weakly degraded, characterized by pH of 6.73, clay 40.3 g kg<sup>-1</sup>, humus 2.32 g kg<sup>-1</sup>, total nitrogen 0.164 g kg<sup>-1</sup>,  $P_{AI}$  18 mg kg<sup>-1</sup>,  $K_{AI}$  210 mg kg<sup>-1</sup>. The research followed the influence of fertilization and harvest phenophase on plants height, the leaves/stems ratio, production of dry matter (DM) per hectare and on feed value (CP – Crude Protein, NDF – Neutral Detergent Fibre, ADF – Acid Detergent Fibre and RFV – Relative Feed Value) in alfalfa at first cut in the second year of vegetation.

It was bi-factorial, 4×3 type experiment, aligned according to the method of subdivided parcels, with the harvestable area of a 15 m<sup>2</sup> plot (3 m × 5 m), in three replicates. The studied factors were: A-fertilization with four treatments ( $a_1$ -unfertilized,  $a_2$ -N<sub>50</sub>P<sub>50</sub>,  $a_3$ -N<sub>75</sub>P<sub>50</sub>

and  $a_4$ -30 Mg ha<sup>-1</sup> manure) and B-harvest period with three treatments ( $b_1$ -early bud,  $b_2$ -late bud and  $b_3$ -full bloom). The manure used had the following composition: 0.445 g kg<sup>-1</sup> N, 0.09 g kg<sup>-1</sup> P and 0.577 g kg<sup>-1</sup> K. The leaves/stems ratio was determined by separating petiole, leaves, buds and flowers from the stem; they were weighed separately and reported as leaves/stems. The DM was determined by drying in drying oven at 105°C for 3 hours. The nitrogen content was determined by the Kjeldahl method, NDF and ADF was determined according to the Van Soest method.

#### **Results and discussion**

Fertilization had a positive influence on height of alfalfa plants on DM production for the whole plant and stems. We found that DM production of leaves was influenced more by the organic fertilization than by the other three variants of fertilization used. The factor with the greatest influence on the productivity of lucerne was the time of harvest. During the advancing of the vegetation period, the production of DM of the whole plant and stems is constantly increasing. The DM production at leaves increases until the end of budding, after which it diminishes. The results showed a decrease in the leaves/stems ratio at all levels of fertilization, especially at the mineral fertilization with  $N_{75}P_{50}$  with delayed harvest (Table 1). In the field, this aspect is easy to see, the alfalfa plants from the mineral fertilized variants being taller, with longer internodes and larger in diameter but with the same number of leaves. These issues were highlighted by other authors, too (Lamb *et al.*, 2003, 2007; Tyrolová and Výborná, 2008).

Expe	erimental plot	Plant height	Dry matte tion (M	er produc- [g ha <sup>-1</sup> )	Leaves/
		(ciii) -	Leaves	Stems	stems ratio
C (11 1	$b_1$ – early bud (control)	45	1.17	2.04	0.57
$a_1 - unfertilized$	$b_2 - late bud$	66*	1.49*	2.86*	0.52°
(control)	$b_{3}^{2}$ – full bloom	81*	1.63*	4.11*	$0.40^{\circ}$
	$b_1 - early bud$	48*	1.54*	3.01*	0.51°
$a_2 - N_{50}P_{50}$	$b_2 - late bud$	71*	1.87*	4.05*	0.46°
2 50 50	b <sub>3</sub> – full bloom	85*	1.69*	4.65*	0.36°
	$b_1 - early bud$	52*	1.51*	3.35*	0.45°
$a_{2} - N_{75}P_{50}$	$b_2 - late bud$	75*	1.72*	4.64*	0.37°
3 /5 50	b <sub>3</sub> – full bloom	88*	1.65*	4.92*	0.33°
- 20 M - h1	$b_1 - early bud$	48*	1.69*	2.70*	0.63*
$a_4 - 50 Mg$ ha <sup>-</sup>	$b_2 - late bud$	72*	1.88*	4.02*	0.47°
manure	$b_3 - full bloom$	84*	1.77*	4.52*	0.39°
	LSD <sub>0.05</sub>	2	0.12	0.33	0.05

Table 1. Influence of fertilization and harvest phenophase on several productivity indicators of alfalfa

The content of crude protein in leaves and stems was not significantly affected by fertilization, but was clearly negatively influenced by harvest, particularly in stems, with values ranging from 17.8 to 11.7 g kg<sup>-1</sup> (Table 2). Delayed harvest strongly increased NDF and ADF in leaves and stems, while the RFV decreased, confirming the results from the literature (Lamb *et al.*, 2007; Petkova and Panayotov, 2007). This results in a decrease in forage value of alfalfa. The increased NDF content in leaves from 25.3 g kg<sup>-1</sup> to 29.9 g kg<sup>-1</sup> and in stems from 60.0 g kg<sup>-1</sup> to 75.9 g kg<sup>-1</sup> resulted in a decrease in digestibility and consumability of the forage.

			Leaves			Stems			Whole	e plant	
Exp.	plot	СР	NDF	ADF	СР	NDF	ADF	CP	NDF	ADF	RFV
_	_		g kg <sup>-1</sup> DN	A	ş	g kg <sup>-1</sup> DM	1		g kg-l	DM	
	b <sub>1</sub>	32.4	25.3	20.3	17.4	60.0	53.1	24.3	47.5	42.3	110
a,	b,	32.5*	26.9*	21.1*	14.9°	64.9*	55.7*	21.3°	51.2*	44.6*	98°
1	b_3	29.2°	28.1*	21.6*	11.7°	73.4*	60.1*	16.7°	60.9*	48.5*	78°
	b,	32.3°	26.3*	21.2*	17.3°	61.1*	53.9*	24.2°	49.6*	44.3*	102°
a,	b,	32.3°	27.3*	21.3*	14.5°	65.4*	56.1*	20.8°	52.9*	46.2*	93°
2	$\tilde{b_3}$	29.5°	29.3*	22.7*	11.8°	71.9*	58.4*	16.4°	63.1*	51.4*	72°
	b <sub>1</sub>	32.5*	27.8*	22.1*	17.8*	60.5*	52.9°	23.2°	50.9*	45.5*	98°
a,	$b_2$	32.4	28.4*	22.6*	14.6°	64.3*	54.9*	20.7°	54.9*	48.0*	87°
3	$\tilde{b_3}$	29.6°	29.9*	23.5*	11.7°	74.0*	60.0*	15.9°	65.2*	52.8*	68°
	b <sub>1</sub>	32.6*	26.6*	21.2*	17.2°	61.4*	54.4*	24.5*	48.1*	43.2*	107°
a,	b,	32.2°	27.2*	21.2*	14.6°	65.7*	56.6*	21.9°	51.8*	45.6*	96°
4	b_3	29.4°	29.3*	22.9*	11.8°	75.9*	62.2*	16.8°	64.1*	51.7*	71°
LSI	D <sub>0.05</sub>	0.1	0,1	0.2	0.1	2	0.2	0.2	0.1	0.1	1

Table 2. Influence of fertilization and harvest phenophase on the quality

CP - crude protein; NDF - neutral detergent fibre; ADF - acid detergent fibre; RFV - Relative Feed Value.

# Conclusions

Fertilization and harvest time positively influenced the height of alfalfa plants, the production of leaves, stems and whole plant, but negatively influenced the leaves/stems ratio by a strong production of stems, compared to the leaves, which negatively influence the forage quality. Delayed harvest has a negative effect on alfalfa forage quality by the decrease of crude protein content and increase of NDF and ADF, particularly in stems, which resulted in a decrease of RFV. Under the conditions studied, we recommend alfalfa to be harvested at the beginning of bud-formation period, regardless the fertilization level, to ensure a good quality of alfalfa forage.

#### References

Delgado I., Andueza D., Muñoz F. and Martínez N. (2001) Effect of nitrogen fertilisation on alfalfa (Medicago sativa L.) regrowth and production. *Options Méditerranéennes. Série A: Séminaires Méditerranéens* 45, 141–143.

Lamb J.F.S., Sheaffer C.C. and Samac D.A. (2003) Population density and harvest maturity effects on leaf and stem yield in Alfalfa. *Agronomy Journal* 95, 635–641.

Lamb J.F.S., Jung H.J.G., Sheaffer C.C., and Samac D.A. (2007) Alfalfa leaf protein and stem cell wall polysaccharide yields under hay and biomass management systems. *Crop Science* 47, 1407–1415.

Oliveira W.S., Oliveira P.P.A., Corsi M., Rodrigo F., Duarte S. and Tsai S.M. (2004) Alfalfa yield and quality as function of nitrogen fertilization and symbiosis with Sinorhizobium meliloti. *Scientia Agricola* 61(4), 433–438.

Petkova D. and Panayotova G. (2007) Comparative study of trifoliolate and multifoliolate alfalfa (*Medicago sativa* L.) synthetic populations. *Bulgarian Journal of Agricultural Science* 13, 221–224.

Rimi F., Macolino S. and Ziliotto U. (2010) Relationships between dry matter yield, forage nutritive value, and some canopy parameters of alfalfa crop. *Grassland Science in Europe* 15, 548–550.

Tyrolová Y. and Výborná A. (2008) Effect of the stage of maturity on the leaf percentage of lucerne and the effect of additives on silage characteristics. *Czech Journal of Animal Science* 53(8), 330–335.

# The effect of manure fertilisation and inoculant treatment on quality and nutritive value of grass silage

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# Abstract

The aim of this study was to evaluate the effect of grassland spring fertilisation with solid manure and herbage treatment with bacterial inoculant on the chemical composition and microflora of grass silage. Three fertilisers were compared: mineral NPK (control), solid manure (50 t ha<sup>-1</sup>) and liquid manure (30 m<sup>3</sup> ha<sup>-1</sup>). Herbage from the first cut of grassland was ensiled in big cylindrical bales with and without addition of bacterial inoculant containing lactic acid bacteria. The content of nutritive components, fermentation products, yeasts, moulds and some bacteria counts in silages were evaluated. There was a significant influence of fertilisation and inoculant treatment on silage quality. Silage made from swards fertilised with manure had significantly higher ammonia concentration and lower content of lactic acid and fatty acids than silages from swards fertilised with NPK. The addition of bacterial inoculants improved quality and nutritive value of all silages. TDN, DMI and RFQ values were higher in inoculated silages than in untreated silages.

Keywords: liquid manure, manure, natural fertilisers, nutritive value, silage

# Introduction

Organic fertilisers of animal origin, i.e. manure, liquid manure and slurry, are important for plant production on many farms, particularly organic farms. Grass from meadows is commonly preserved by making silage. From a few recent studies it appears that grassland fertilisation with natural fertilisers may affect the ensiling process and consequently the quality of obtained silage and indirectly the quality of produced milk. Particularly negative effect on the ensiling process and the quality of silage is exerted by non-fermented manure applied in large doses and on improper terms (Rammer *et al.*, 1994; Davies *et al.*, 1996; Rammer and Lingvall, 1997; Johansen and Todnem, 2002; Pauly and Rodhe, 2002). The aim of this study was to evaluate the effect of grassland spring fertilisation with solid manure and herbage treatment with bacterial inoculant on the chemical composition and microflora of grass silage.

#### Materials and methods

Studies were carried out in 2011 in a plot experiment established in 2006 on a permanent meadow situated on mineral soil of the Experimental Farm in Falenty. Three plots of an area of 0.6 ha each were fertilised as follows: plot 1 – mineral NPK fertiliser (N: 120 kg ha<sup>-1</sup>, P: 60 kg ha<sup>-1</sup>, K: 120 kg ha<sup>-1</sup>), plot 2 – solid manure 50 t ha<sup>-1</sup>, plot 3 – liquid manure 60 m<sup>3</sup> ha<sup>-1</sup> + 18 kg ha<sup>-1</sup> of mineral P. Nitrogen and potassium as mineral fertilisers were applied in three equal doses in spring, after the first and second cut and P once in spring. Manure (20% DM) was applied in March 2011, after 6 months of storage on a manure slab. Liquid manure (4% DM) was applied in two equal doses (30 m<sup>3</sup> ha<sup>-1</sup>) in April 2011 and after the first cut (end of May 2011). Application to the soil was by shallow injection (to 20 mm) with applicators spanned by 15 cm. The doses of manure and liquid manure were calculated according to chemical analyses of fertilisers. The doses of manure (M) and liquid manure (LM) were equivalent to approximately the 120 kg N that was applied in the control (NPK).

In 2011, the meadow sward comprised 82–84% grasses, 2–5% of legumes and 9–11% weeds and herbs. Among the dominant group of grasses were *Alopecurus pratensis* L. (37–50%), *Poa pratensis* L. (12–17%), *Lolium multiflorum* Lam. (9–18%) and *Poa trivialis* L. (species present only on the treatment with liquid manure fertilisation in amount of 10%). The botanical composition of meadow sward on treatments was affected by the fertilisation applied in the same form and dose since 2006.

Experimental plots were cut on 20 May 2011. Mown grass after pre-wilting on the meadow surface was collected with the rolling press and ensiled in big bales. Three big bales from each fertilisation plot were ensiled without and three with addition of bacterial inoculant containing two strains of Lactobacillus plantarum, L. brevis, L. buchneri and two enzymes. In November, two silage samples from each big bale were taken for chemical analyses. Dry matter (DM) (oven method at 105°C) and pH of fresh mass (potentiometric method) were determined. The samples of silage were analysed for lactic acid (LA), volatile fatty acids (VFA), ammonia, crude protein, WSC, crude ash, crude fibre and its fractions: neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) content. Analyses were made using the near infrared spectroscopy (NIRS technique) using NIRFlex N-500 spectrophotometer using calibrations prepared for dry forages and wet fermented forages by the firm INGOT<sup>R</sup>. Nutritive value of silage was expressed as an index of relative forage quality (RFQ) (Undersander and Moore, 2002). In addition, the total number of aerobic bacteria, Enterobacteriaceae, number of yeasts and moulds (cultures on PetrifilmTM 3M plates) in fresh silage samples (FM) were determined. Data concerning the chemical and biological composition of silage were analysed using analysis of variance in ANOVA 1. Differences between treatments were tested using the Student's t-test.

#### Results

Applied fertilisation had a significant effect on fermentation parameters of examined silage samples. Fertilisation with manure exerted the most unfavourable effect on the silage quality. Silage from the sward fertilised with manure showed significantly higher ammonia concentration, lower content of fermentation products with a significantly lower content of LA and VFA in the dry mass of silage than silages from treatments fertilised with NPK. The fertilisation with liquid manure had less unfavourable influence on fermentation parameters. The content of ammonia and LA was similar and VFA even lower in silage from meadow sward fertilised with liquid manure than in silage from plot fertilised with NPK fertilisers. Fertilisation also had a significant impact on the content of nutritive components in analysed forage samples. In relation to NPK fertilisation, silages from the treatment fertilised with natural fertilisers showed higher content of crude protein and crude ash and lower content of crude fibre (particularly silages from fields fertilised with liquid manure). Applied fertilisation exerted also a significant effect on total number of aerobic bacteria, Enterobacteria and moulds (Table 1). The addition of bacterial inoculants containing selected lactic acid bacteria and enzymes to the ensiled herbage improved quality of all silages. Silages made of herbage with addition of bacterial inoculant had lower pH level, lower ammonium content and higher lactic acid content. Inoculant treatment (LAB + enzymes) had also an evident impact on the nutritive value of the feeds. TDN, DMI and RFQ values were higher in inoculated silages than in untreated silages. In spite of high single dose of natural fertilisers (50 t ha<sup>-1</sup> manure and 30 m<sup>3</sup> ha<sup>-1</sup> liquid manure) the unfavourable influence on fermentation process and silage quality was less evident than was observed in previous years of study (Wróbel and Jankowska-Huflejt, 2010). The explanation of this result can be fact the solid manure was composted before application. Also the time between application and harvest was long enough to avoid the risk of crop contamination with detrimental organisms.

	NF	РК	Mar	nure	Liquid 1	manure	
	Untreated	LAB	Untreated	LAB	Untreated	LAB	
DM (g kg <sup>-1</sup> )	426.7a	470.0cd	430ab	435ab	457.1bc	490.0d	
pH	4.53a	4.66b	4.61ab	4.60ab	4.82c	4.65ab	
$NH_3-N$ (g kg <sup>-1</sup> total N)	78.6bc	68.0b	80.3c	85.1c	68.6b	56.3a	
$LA (g kg^{-1} DM)$	24.17ab	32.11bc	22.61a	31.98abc	33.90cd	42.34d	
VFA (g kg <sup>-1</sup> DM)	46.57c	38.70bc	29.92a	36.69ab	28.45a	29.24a	
The sum of FP (g kg <sup>-1</sup> DM)	70.74b	70.81b	52.53a	68.67b	62.35ab	71.59b	
The share of LA in the sum of FP (%)	33.80a	45.29ab	43.81ab	45.80abc	54.00bc	58.41c	
Crude protein (g kg <sup>-1</sup> DM)	111.3a	109.7a	112.7ab	115.8b	115.5b	116.8b	
Crude fiber (g kg <sup>-1</sup> DM)	300.0d	294.2cd	285.9c	286.4c	271.6b	255.8a	
Crude ash (g $kg^{-1}$ DM)	79.2b	73.2a	89.6d	84.4c	92.7d	89.0d	
WSC (g kg <sup>-1</sup> DM)	92.1ab	101.7bc	87.0a	91.0a	109.6c	128.3d	
NDF (g kg <sup><math>-1</math></sup> DM)	523.9d	510.5c	504.9bc	497.6b	500.7bc	480.7a	
ADF (g kg <sup>-1</sup> DM)	338.0c	343.2c	336.9c	336.4c	319.3b	306.3a	
ADL (g kg <sup>-1</sup> DM)	38.6b	44.5c	38.5b	40.4b	32.2a	30.0a	
TDN (% DM)	63.42ab	64.52c	63.08a	63.83b	62.91a	63.99bc	
DMI (% body weight)	2.87a	2.92c	2.87a	2.92bc	2.88ab	2.95c	
RFQ	148a	153b	147a	152b	148a	154b	
Total number of aerobic bacteria	7.601	7 104	672ah	6 70ab	5 600	5710	
$(\log_{10} \text{cfu g}^{-1} \text{FM})$	7.090	7.160	0.72a0	0.70a0	J.08a	3./1a	
<i>Enterobacteria</i> (log <sub>10</sub> cfu g <sup>-1</sup> FM)	2.58ab	1.83a	4.01b	1.91a	2.80ab	2.70ab	
Yeasts $(\log_{10} \text{cfu g}^{-1} \text{FM})$	2.53b	1.72ab	1.54a	1.20a	1.30a	1.23a	
Moulds $(\log_{10} \text{ cfu } \text{g}^{-1} \text{ FM})$	4.58ab	4.60ab	4.91b	3.37a	5.36b	4.50ab	

Table 1. Fermentation quality, nutritive value and microflora of silage

DM - dry matter; WSC - water soluble carbohydrates; NDF - neutral detergent fibre; ADF - acid detergent fibre; ADL - lignin; TDN - total digestible nutrients; DMI - dry matter intake; RFQ - Relative Forage Quality =  $DMI \times TDN/1.23$ ; LA - lactic acid; VFA - volatile fatty acids; FP - fermentation products; VFA = acetic + butyric + propionic acid; Values with different letters are significantly different (P < 0.05).

#### Conclusions

A significant influence of natural fertilisation on some chemical and microbiological parameters was reported. The most unfavourable impact on silage quality and on some selected parameters of microbial evaluation was fertilisation with solid manure. A good way of improvement of silage quality made from swards fertilised with manure and liquid manure was through the addition of bacterial inoculants containing selected lactic acid bacteria.

#### References

Davies D.R., Merry R.J. and Bakewell E.L. (1996) The effect of timing of slurry application on the microflora of grass and changes occurring during silage fermentation. *Grass and Forage Science* 51, 42–51. Johansen A. and Todnem J. (2002) Effects of cattle and sheep slurry on grass silage quality. In: Conference Proc. the XIIIth International Silage Conference September 11–13, 2002, Auchincruive, Scotland, 408–409. Pauly T.M. and Rodhe L. (2002) Slurry application on ley – Effect of application method on the hygienic quality of grass silage. In: Conference Proc. the XIIIth International Silage Conference September 11–13, 2002, Auchincruive, Scotland, 410–411.

Rammer C. and Lingvall P. (1997) Influence of farmyard manure on the quality of grass silage. *Journal of the Science of Food and Agriculture* 75, 133–140.

Rammer C., Östling C., Lingvall P. and Lindgren S. (1994) Ensiling of manured crops – effects on fermentation. *Grass and Forage Science* 49, 343–351.

Undersander D. and Moore J.E. (2002) Relative forage quality. UW Extention. *Focus on Forage* 4, 5. Wróbel B. and Jankowska-Huflejt H. (2010) The influence of natural fertilisation on quality and nutritive value of grass silage. *Grassland Science in Europe* 15.

# Fatty acid composition of three different grassland species

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# Abstract

The botanical composition and the growth stage of forage influence the milk fatty acid profile. In an experiment, the fatty acids composition of the three grassland species *Dactylis glomerata*, *Medicago sativa* and *Taraxacum officinale* was investigated. Forage of the first cut was harvested at four different dates, and different plant parts – stems, leaves and flowers – were collected separately and analysed. The samples were freeze-dried and the fatty acids determined by gas chromatography. In addition, the nutrient contents were analysed. The  $\alpha$ -linolenic acid (C 18:3 c9c12c15) was the most dominant fatty acid for *D. glomerata* and *M. sativa*, though for *T. officinale* this was the case only in the early growth stage. In the older forage, more linoleic acid (C 18:2 c9c12) was produced.

For all three species, total fatty acids decreased from the first to the fourth cutting date. The leaves and flowers contained more fatty acids than the stems. The biggest differences between stems and leaves were found for *D. glomerata*. The highest contents of linoleic acid (C 18:2 c9c12) were found in the flowers of *T. officinale*.

Keywords: grassland species, growth stage, fatty acids, linolenic acid, linoleic acid

# Introduction

The botanical composition and growth stage of forage influence the milk fatty acid profile (Ferlay *et al.*, 2006). There are several fatty acids in plants, the most important of which are  $\alpha$ -linolenic acid, linoleic acid and palmitic acid (Bauchart *et al.*, 1984). Wyss and Collomb (2010) and Warner *et al.* (2010) found differences in the fatty acid concentration of different grasses, legumes and herbs. The different fatty acids decreased with the age of the forage, especially in the forage of the first growth.

The objective of this study was to investigate the fatty acid concentration in different plant parts of three different grassland species at different growth stages.

# Materials and methods

The fatty acid composition of the grass *Dactylis glomerata*, the legume *Medicago sativa* and the herb *Taraxacum officinale* was investigated. All species were grown at Posieux (altitude 650 m a.s.l.). Forage of the first cut was harvested at four different dates. In addition, the different plant parts – stems, leaves and flowers – were collected separately and analysed, although the three plant species had not always developed stems and flowers at all the harvested dates. The samples were freeze-dried and the fatty acids were determined by gas chromatography (Alves *et al.*, 2008). In addition, for the whole plants, samples were dried at 60°C, milled, and the ash, crude protein and fibre contents were analysed. Results for fatty acid concentrations were analysed by analysis of variance.

#### **Results and discussion**

The nutrient contents of the three different grassland species are shown in Table 1. With increasing age of the plants the crude protein (CP) decreased and the fibre contents, acid detergent fibre (ADF) and neutral detergent fibre (NDF), increased.

Species	Cutting data -	DM	Ash	СР	ADF	NDF
	Cutting date -	g kg <sup>-1</sup>	g kg <sup>-1</sup> DM			
Dactylis glomerata	03.05.	226	96	206	252	427
	17.05.	213	74	212	253	427
	31.05.	252	51	137	277	498
	14.06.	260	48	95	375	611
Medicago sativa	03.05.	185	89	239	200	277
	17.05.	226	78	202	269	312
	31.05.	300	68	155	335	414
	14.06.	262	82	151	357	431
Taraxacum officinale	20.04.	154	106	235	179	191
	03.05.	110	90	162	202	206
	17.05.	116	83	144	204	214
	31.05.	127	118	111	251	258

Table 1. Nutrient contents in three species and four cutting dates of the first growth

The total fatty acids (TFA),  $\alpha$ -linolenic acid, linoleic acid and palmitic acid were significantly different between the three species and cutting dates (Table 2). The highest fatty acid concentrations were found for all three plants in the young forage. The herb *T. officinale* always had higher concentrations of linoleic (C18:2 c9,c12) and palmitic acid (C16:0) in comparison with *D. glomerata* and *M. sativa*. Bauchart *et al.* (1984) also found the highest fatty acid concentrations in early May. The results were similar to the results of an earlier study (Wyss and Collomb, 2010).

Table 2. Fatty	acid concent	rations in thre	e species a	and four	cutting of	dates of t	he first	growth
$(g kg^{-1} DM)$								

Species	Cutting date	C16:0	C18:2 c9,c12	C18:3 c9c12c15	TFA
Dactylis glomerata	03.05.	4.3	4.1	20.1	34.6
	17.05.	4.2	4.4	20.4	34.5
	31.05.	3.6	4.5	15.4	28.1
	14.06.	3.0	3.3	10.7	21.1
Medicago sativa	03.05.	5.1	6.8	17.3	34.8
	17.05.	5.0	6.7	15.3	32.2
	31.05.	4.1	5.1	12.0	25.6
	14.06.	3.8	4.6	10.8	23.4
Taraxacum officinale	03.05.	6.8	10.0	16.4	39.2
	17.05.	5.6	12.9	16.0	40.0
	31.05.	4.4	10.4	9.2	28.9
Standard deviation		0.34	0.73	1.14	1.48
Species (S)		***	***	***	***
Cutting date (D)		***	***	***	***
Interaction $S \times D$		***	***	***	***

\*\*\* *P* < 0.001.

The separation of the different plant parts showed that the concentration of the fatty acids strongly varied within the plants (Table 3). For *D. glomerata* big differences were found between the stems, as compared with the leaves and flowers. For *M. sativa* and *T. of-ficinale* differences were also found between the different plant parts, but the differences were smaller than in *D. glomerata*. A high accumulation of linoleic acid was found in the flowers of *T. officinale*.

Species	Plant part	Cutting date	C16:0	C18:2 c9c12	C18:3 c9c12c15	TFA
Dactylis glomerata	Stem	31.05.	2.1	1.5	2.3	7.1
		14.06.	1.5	1.7	3.0	7.2
	Leaves	31.05.	3.9	3.7	20.8	34.2
		14.06.	3.6	3.6	15.0	28.2
	Bloom	31.05.	3.8	7.0	8.9	25.1
		14.06.	5.4	4.3	13.9	30.8
Medicago sativa	Stem	03.05.	4.5	8.3	7.7	22.9
		17.05.	4.1	8.1	7.3	22.3
		31.05.	3.1	5.8	5.7	16.7
		14.06.	2.5	4.0	4.1	12.2
	Leaves	03.05.	6.1	6.3	24.7	45.0
		17.05.	5.6	6.0	22.7	41.4
		31.05.	5.5	5.5	23.5	41.3
		14.06.	5.2	4.9	20.1	37.4
	Bloom	14.06.	6.5	6.1	10.7	28.8
Taraxacum officinale	Stem	03.05.	5.2	9.8	8.7	25.9
		17.05.	4.2	7.4	7.3	20.7
		31.05.	3.4	6.1	4.9	17.1
	Leaves	03.05.	6.1	7.9	22.5	42.6
		17.05.	5.6	8.3	22.7	42.4
		31.05.	5.1	7.2	14.5	32.6
	Bloom	03.05.	10.4	17.7	15.5	54.4
		17.05.	7.9	26.6	8.2	54.0
		31.05.	6.3	15.7	4.1	35.7
Standard deviation			0.88	2.18	2.95	4.97
Species (S)			***	***	ns	***
Plant parts (P)			***	***	***	***
Interaction $S \times P$			ns	***	ns	**

Table 3. Fatty acid concentrations in the different plant parts of three species (g kg<sup>-1</sup> DM)

\*\* *P* > 0.01; \*\*\* *P* < 0.001; ns – non significant.

#### Conclusions

 $\alpha$ -linolenic acid is the dominant fatty acid in the three investigated grassland species. Forage age is a important factor for the fatty acid concentration in the different plants. The concentrations of the different fatty acids vary between the different plant parts.

#### References

Alves S.P., Cabrita A.R.J., Fonseca A.J.M. and Bessa R.J.B. (2008) Improved method for fatty acid analysis in herbage based on direct transesterification followed by solid-phase extraction. *Journal of Chromatography* 1209, 212–219.

Bauchart D., Verite R. and Remond B. (1984) Long-chain fatty acid digestion in lactating cows fed fresh grass from spring to autumn. *Canadian Journal of Animal Science* 64 (Suppl.), 330–331.

Ferlay A., Martin B., Pradel P. Coulon J.P. and Chilliard Y. (2006) Influence of grass-based diets on milk fatty acid composition and milk lipolytic system in Tarentaise and Montbeliarde cow breeds. *Journal of Dairy Science* 89, 4026–4021.

Warner D., Jensen S.K., Cone J.W. and Elgersma A. (2010) Fatty acid composition of forage herb species. *Grassland Science in Europe* 15, 491–493.

Wyss U. and Collomb M. (2010) Fatty acid composition of different grassland species. *Grassland Science in Europe* 15, 631–633.

# Session 2.2.

"Green" products in the food chain

# Effects of fresh forage inclusion in diet of high performance dairy cows on milk production and composition

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# Abstract

The aim of this research was to evaluate the effects of partial substitution of total mixed ration (TMR) with fresh forage in the diet of high performance dairy cows, on milk production and composition. Two trials were performed, comparing a cow diet based on TMR with high percentage of maize silage and concentrates (TMR) with one where cows were fed TMR plus freshly harvested forage (TMR+F). In Trial 1 the fresh forage was utilized during an advanced stage of maturity, whereas in Trial 2 the fresh forage in the cow diet did not affect the milk production and its contents of protein and fat. The fresh forage affected the milk fatty acid (FA) composition, increasing the vaccenic and linolenic acids and conjugated linoleic acid (CLA) contents. A supplement of fresh forage to TMR can modify the nutritional properties of cow milk without changing the milk production.

Keywords: TMR, fresh forage, cow, milk, fatty acid composition

#### Introduction

Even if fresh forage is a valuable feed resource for dairy farms, in Italy it is not utilized in the diets of high performance dairy cows. They are often fed with total mixed rations (TMR) mainly based on maize silage and concentrates, which meet the nutritional requirements for high milk production. However, milk produced from diets based on maize silage and concentrates has a high proportion of saturated fatty acid (SFA), which has negative effects on human health (Dewhurst *et al.*, 2006). Conversely, an increased proportion of fresh forage in the diet allows milk to be obtained with a FA profile more favourable to human health, due to increasing values of polyunsaturated FA (PUFA), omega-3 FA and conjugated linoleic acid (CLA) (Dewhurst *et al.*, 2006; Morales-Alvaràz *et al.*, 2010). The aim of this research was to evaluate the effects of a partial substitution of total mixed ration (TMR) with fresh forage in the diet of high performance dairy cows, consistent with milk production and composition.

#### Materials and methods

Eight primiparous Italian Friesian cows were involved in two trials. In both trials, cows were allocated into two groups, each of four animals, according to body weight, dry matter intake (DMI; 19.4±0.63 kg d<sup>-1</sup>), milk production (32.2±3.1 kg d<sup>-1</sup>), and day in milking (171±18 d). In both trials, the first group (TMR) received a TMR (37.7% of maize silage, 14.8% of hay and 47.4% of concentrates on DM basis) offered *ad libitum*. The TMR contained the following contents of crude protein (CP), NDF, ADF and net energy (NE<sub>1</sub>): 140 g kg<sup>-1</sup> DM, 312 g kg<sup>-1</sup> DM, 175 g kg<sup>-1</sup>

DM and 1.56 Mcal kg<sup>-1</sup> DM, respectively. The second group (TMR+F) was fed TMR previously described, offered *ad libitum*, plus 30 kg cow<sup>-1</sup> d<sup>-1</sup> of freshly harvested forage from a meadow dominated by grass species (*Lolium multiflorum* more than 70% of DM). In Trial 1 the fresh forage was utilized during an advanced stage of maturity and had a DM of 238 g kg<sup>-1</sup> of fresh matter (FM), 132 g kg<sup>-1</sup> DM of CP, 523 g kg<sup>-1</sup> DM of NDF, 315 g kg<sup>-1</sup> DM of ADF, and 1.36 Mcal kg<sup>-1</sup> DM of NE<sub>L</sub>. In Trial 2 the fresh forage was utilized during an early stage of maturity and had a DM of 198 g kg<sup>-1</sup> of FM, 154 g kg<sup>-1</sup> DM of CP; 480 g kg<sup>-1</sup> DM of NDF, 288 g kg<sup>-1</sup> DM of ADF and 1.44 Mcal kg<sup>-1</sup> DM of NE<sub>L</sub>. Each trial lasted for 21 d. After fourteen days of adaptation, the DMI and milk yield were recorded daily during the experimental period. TMR, fresh forage offered and refused, and milk samples were collected daily. TMR and fresh forage were dried for DM analysis, ground and analysed for their chemical composition. Milk samples were analysed for fat and protein contents by MilkoScan FT6000, and for FA composition by gas—chromatography. Statistical analysis was performed in SPSS 16.0 using one-way ANOVA procedure (*P* < 0.05).

Table 1. Total dry matter intake (DMI), total mixed ration (TMR), net energy  $(NE_L)$  and protein intakes of dairy cows during the two experimental trials

	<u> </u>							
	Trial 1				Trial 2			
	TMR	TMR+F	SEM	P-value	TMR	TMR+F	SEM	P-value
Intake								
DM (kg DM $cow^{-1} d^{-1}$ )	19.96	20.98	0.644	0.459	21.33	21.49	0.236	0.752
TMR (kg DM $cow^{-1} d^{-1}$ )	19.96	15.39	0.900	0.002	21.33	16.15	0.845	< 0.001
Forage (kg DM cow <sup>-1</sup> d <sup>-1</sup> )	_	5.59	_	_	_	5.34	_	_
$NE_r$ (kg DM cow <sup>-1</sup> d <sup>-1</sup> )	31.3	31.7	0.945	0.833	33.1	32.7	0.364	0.670
Protein (kg DM cow <sup>-1</sup> d <sup>-1</sup> )	2792	2891	87.2	0.600	2967	3069	37.0	0.182

#### **Results and discussion**

In both trials the DM, NE<sub>L</sub> and protein intakes did not differ between treatments (Table 1) and the fresh forage accounted for 26.6 and 24.8% of DMI in Trial 1 and 2, respectively. In both trials, the diets did not affect the milk yield, milk fat or protein concentrations (Table 2). However, in Trial 1 milk yield and milk protein contents were numerically lower for TMR+F than TMR, likely due to the low nutritional quality of fresh forage. In both trials, the milk FA composition was affected by diets, and higher milk contents of CLA and vaccenic acid (C18:1 *t*11) were observed with the TMR+F diet than the TMR diet (Table 2). The fresh forage harvested at an early stage of maturity (Trial 2) allowed milk to be obtained that had a lower content of SFA and higher contents of oleic (C18:1 *c*9) and linolenic (C18:3 *c*9, *c*12, *c*15) acids, MUFA, and odd and branched chain FA (OBCFA) compared with milk from cows fed TMR only. These results are in agreement with other studies, where the supplement of fresh forage to TMR affects positively the quality of cow milk fat, leading to a FA profile more favourable for human health (Bargo *et al.*, 2006; Morales-Alvaràz *et al.*, 2010). The different response to fresh forage introduction on milk FA profile observed during these two trials could be due to the different maturity stage of forage (Dewhurst *et al.*, 2006).

#### Conclusions

The inclusion of fresh forage in cow diets could be considered as a means of producing healthier milk from high performance dairy cows. Results showed that a supplement of fresh forage to TMR can improve the FA profile of milk and increase its CLA and omega-3 FA contents without modifying the quantity of produced milk, especially when herbage is harvested at early stage of maturity.

I I I I I I I I I								
	Trial 1				Trial 2			
	TMR	TMR+F	SEM	P-value	TMR	TMR+F	SEM	P-value
Milk yield (kg cow <sup>-1</sup> d <sup>-1</sup> )	33.2	29.5	1.175	0.119	31.8	30.4	0.974	0.476
Fat content (g kg <sup>-1</sup> )	35.4	35.8	0.100	0.839	35.6	37.4	0.099	0.373
Protein content (g kg <sup>-1</sup> )	33.7	31.6	0.055	0.058	33.2	33.3	0.052	0.923
Fatty acids (g 100g <sup>-1</sup> FA)								
C4:0	3.79	3.76	0.126	0.912	4.25	3.71	0.078	< 0.001
C6:0	2.19	2.32	0.047	0.146	2.58	2.09	0.061	< 0.001
C8:0	1.24	1.29	0.033	0.498	1.44	1.15	0.040	< 0.001
C10:0	2.79	2.71	0.085	0.626	3.07	2.54	0.089	0.001
C10:1	0.24	0.24	0.010	0.896	0.32	0.22	0.012	< 0.001
C12:0	3.09	2.88	0.084	0.237	3.36	2.82	0.091	0.001
C14:0	10.34	9.85	0.192	0.206	10.89	10.15	0.205	0.067
C14:1	0.80	0.77	0.030	0.587	0.94	0.81	0.026	0.007
C15:0	0.88	0.80	0.025	0.126	0.88	0.92	0.014	0.091
C15.1	0.03	0.03	0.001	0.214	0.03	0.04	0.001	< 0.001
C16:0	27.99	29.10	0.334	0.100	31.08	27.96	0.416	< 0.001
C16:1	1.40	1.65	0.045	0.001	1.54	1.66	0.057	0.299
C17:0	0.46	0.47	0.012	0.677	0.41	0.56	0.020	< 0.001
C17:1	0.20	0.23	0.008	0.020	0.17	0.29	0.015	< 0.001
C18:0	11.45	10.83	0.212	0.151	10.00	10.87	0.245	0.075
C18:1 <i>c</i> 9	24.12	23.28	0.515	0.434	20.63	24.98	0.589	< 0.001
C18:1 <i>t</i> 11	0.69	1.03	0.075	0.015	0.79	1.32	0.078	< 0.001
C18:1(t/c15/16)	1.89	2.23	0.101	0.089	1.72	1.40	0.056	0.001
C18:2 <i>c</i> 9 <i>c</i> 12	2.82	2.90	0.111	0.707	2.48	2.33	0.040	0.065
C18:2 <i>t</i> 9 <i>t</i> 12	0.16	0.19	0.006	0.043	0.17	0.25	0.012	$<\!0.001$
C18:3 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15	0.29	0.34	0.015	0.079	0.31	0.39	0.010	< 0.001
C18:2 <i>c</i> 9 <i>t</i> 11 CLA	0.38	0.50	0.027	0.028	0.37	0.48	0.017	< 0.001
C20:0	0.20	0.19	0.005	0.189	0.21	0.23	0.005	0.044
C20:1	0.23	0.22	0.005	0.384	0.25	0.27	0.006	0.029
SFA	66.64	66.27	0.668	0.793	70.17	65.41	0.650	< 0.001
MUFA	29.70	29.79	0.571	0.939	26.50	31.13	0.635	< 0.001
PUFA	3.66	3.94	0.149	0.371	3.33	3.46	0.045	0.152
OBCFA	3.83	3.67	0.044	0.061	3.54	4.32	0.102	< 0.001

Table 2. Milk production, milk fat and protein contents and milk fatty acid profile during the two experimental trials

CLA, conjugated linoleic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; OBCFA, sum of odd and branched chain fatty acids.

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#### References

Bargo F., Delahoy J.E., Schroeder G.F., Baumgard L.H. and Muller L.D. (2006) Supplementing total mixed rations with pasture increase the content of conjugated linoleic acid in milk. *Animal Feed Science and Technology* 131, 226–240.

Dewhurst R.J., Shingfield K.J., Lee M.R.F. and Scollan N.D. (2006) Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology* 131, 168–206.

Morales-Almaráz E., Soldado A., González A., Martínez-Fernández A., Domínguez-Vara I., De la Roza--Delgado B. and Vicente F. (2010) Improving the fatty acid profile of dairy cow milk by combining grazing with feeding of total mixed ration. *Journal of Dairy Research* 77, 225–230.

# Effect of pasture type compared with hay diet on dairy goat milk production and quality

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#### Abstract

The objective was to examine the effect of pasture type, and of grazing compared with hay feeding, on milk production and quality from dairy goats in early and late grazing season. Eighty goats were grouped according to genotype and lactation, and randomly divided into two groups with approximately 8 weeks difference in kidding date and start of feeding experiment, in Early and Late grazing season. At the start of the feeding experiment the goats were divided into 4 forage-treatment groups: R, forest rangeland pasture; C, cultivated pasture; HH, high quality hay; HL, low quality hay. Group R yielded less milk (1.5 vs. 2.0 kg d<sup>-1</sup>) and lower milk protein content (32 vs. 33 g kg<sup>-1</sup>), but higher milk fat (46 vs. 37 g kg<sup>-1</sup>) and DM content (120 vs. 113 g kg<sup>-1</sup>) than group C. Free fatty acids (FFA) content in milk was not affected by pasture type. The effects of pasture type on milk yield and milk constituents were similar in early and late grazing season. Grazing resulted in similar milk yield but higher milk fat (42 vs. 34 g kg<sup>-1</sup>), protein (32 vs. 30 g kg<sup>-1</sup>) and DM (117 vs. 106 g kg<sup>-1</sup>) content, and lower content of FFA (0.22 vs. 0.34 mEq L<sup>-1</sup>) than hay feeding.

Keywords: forest grazing, pasture, hay, goats, milk yield, milk composition

#### Introduction

Dairy goat milk quality in Norway is variable and often does not meet the requirements of the industry for cheese making. The main problems are lipolysis and high content of free fatty acids (FFA) causing tart and rancid off-flavour. High frequencies of the  $\alpha_{1}$  – casein "null" variant in the milk from Norwegian dairy goats is an important reason for the inferior milk quality, including high FFA and low fat and protein contents (Chilliard et al., 2003; Devold et al., 2011). However, reduced pasture allowance and decreased herbage quality on forest or mountain pastures with underfeeding and negative energy balance as a consequence are also believed to be major causes of the problem with FFA, as the FFA content of milk usually increases during the grazing season (Eknæs et al., 2006). At the same time it is known that the FFA content of milk is highest during mid-lactation, which coincides with the time when goats are at pasture (Chilliard et al., 2003). Therefore, the season effect on milk quality is confounded by the effect of lactation stage. In the present study we tested if production and quality of milk of dairy goats at the same stage of lactation are affected by the type of pasture; i.e. rangeland compared with cultivated pastures, or by grazing compared with hay feeding. Additionally, we tested whether there were grazing-season effects on the same production traits.

#### Materials and methods

Eighty dairy goats, at Senja, Norway (N 69°21.397', E 17°56.319'), were grouped according to genotype and lactation number, and randomly divided into two groups (Early and Late) with approximately 8 weeks difference in kidding. From kidding until start of the grazing season, at the end of June 2010, the goats received the same diet. At the start of the grazing season the 40 Early goats were grouped similarly, according to genotype and lactation number, and randomly divided into 4 forage treatment groups: C = cultivated pasture, R = forest heterogeneous rangeland pasture, HH = High quality hay [Net energy lactation (NEL) =  $5.5 \text{ MJ kg}^{-1}$ ; Crude protein (CP) = 197gkg<sup>-1</sup> DM], HL = low quality hay (NEL = 4.7 MJ kg<sup>-1</sup>; CP = 109 g kg<sup>-1</sup> DM). The hay was fed ad libitum. The cultivated pasture (C) was a ley in its first production year, dominated by Phleum pratense and Festuca pratensis. Characterization of the R pasture is given by Jørgensen et al. (2012; these proceedings). The Late group grazed together with the R group of the Early goats until the middle of August, when they also were randomly allocated in the same way to the 4 forage treatment groups. The goats were supplemented with a concentrate mixture of 0.9 kg DM goat<sup>-1</sup> d<sup>-1</sup>. The forage-treatment periods lasted for 3 weeks. Milk yield was measured and milk samples were collected during three periods: the indoor period (1), the week before they were allocated to the diet treatments (2), and during the last week of the diet treatment period (3). The milk samples were analysed by Fourier transform infrared (Foss Electric Milkoscan FT 6000, Foss Electric, Hillerød, Denmark). Animal variables were analysed using a mixed model in SAS (SAS, 2008). Diet treatment (C, R, HH and HL), grazing season (Early and Late) and period (1, 2 and 3) and their interactions were included in the model with period treated as repeated measure and goat as the subject. Animal was classified as a random effect. Orthogonal contrasts were used to separate treatments means in the diet-treatment period (3).

#### **Results and discussion**

Milk yield (1.2 vs. 2.3 g kg<sup>-1</sup>, P < 0.001) and milk FFA content (0.22 vs. 0.34 mEq L<sup>-1</sup>, P < 0.05) was on average lower in Late than in Early grazing season (Table 1). The seasonal effect on other milk constituents depended on forage type: milk fat, protein, lactose, DM and urea decreased from Early to Late season in goats at grazing, and increased in goats on hay diets. However, the seasonal effect is also an effect of pre-experimental diets, as the goats in 'Early' went directly from an indoor silage-based diet to the experimental diets, while the goats in 'Late' grazed for about 8 weeks in the forest before being subjected to the dietary treatments. Goats grazing in forest (R) yielded on average less milk (1.5 vs. 2.0 kg d<sup>-1</sup>, P < 0.01) and lower milk protein (32 vs. 33, g kg<sup>-1</sup>, P < 0.05) and urea content (6.7 vs. 10.4 mmol L<sup>-1</sup>, P < 0.001), but higher milk fat (46 vs. 37 g kg<sup>-1</sup>, P < 0.001) and DM content (120 vs. 113 g kg<sup>-1</sup>, P < 0.001) than those grazing cultivated pasture (Table 1). The effects of pasture type on milk yield and milk constituents were similar in early and late grazing seasons. The effect of pasture type on milk yield and constituents was likely to be due to lower energy and protein intake on R than on C (Santini et al., 1992; Dønnem et al., 2011a), and more energy expenditure on R due to locomotion (Lachica et al., 1999). Grazing gave, on average, similar milk yield, but higher milk fat (42 vs. 34g kg<sup>-1</sup>, P < 0.001), protein (32 vs. 30 g kg<sup>-1</sup>, P < 0.001) and DM (117 vs. 106 g kg<sup>-1</sup>, P < 0.001) content and thus higher yield of energy corrected milk (1.7 vs. 1.5 kg d<sup>-1</sup>, P < 0.05) than hay. Goats on pasture produced, on average, milk with lower FFA (0.22) vs. 0.34, mEq L<sup>-1</sup>, P < 0.05) and urea (8.5 vs. 10.4 mmol L<sup>-1</sup>, P < 0.001) content than hay. With respect to milk protein and urea content, goats on pasture probably had a lower intake of fibre but higher intake of energy than goats on hay diets. Higher energy intake may also explain higher milk fat content from pasture than for hay (Dønnem et al., 2011b), even though the fibre intake was likely to be higher on hay than on pasture. Negative energy balance and mobilization of body
fat may also contribute to increased milk fat. The goats in the present study lost weight during the feeding experiment and probably mobilized body fat, but there were no difference in weight change between treatments. Mobilization of body fat may explain the relatively low content of FFA on all treatments in the present experiment (Dønnem *et al.*, 2011b).

			0			·			-	-	
	See	Pasture	type (P)	Hay qua	ality (H)			Si	ignifican	ce	
Item	son (S)	С	R	HH	HL	SEM	S	Cvs.R	Pvs.H	Svs.P	Svs. Pvs.H
Milk,	Early	2.45	1.88	2.65	2.16	0.174	***	**	ns	ns	ns
kg d <sup>-1</sup>	Late	1.50	1.15	1.38	0.83						
Milk fat,	Early	39.9	49.8	32.3	34.6	1.81	ns	***	***	ns	**
g kg <sup>-1</sup>	Late	34.2	42.9	33.4	36.8						
Milk protein,	Early	33.3	31.7	29.1	29.6	0.53	ns	*	***	ns	*
g kg <sup>-1</sup>	Late	32.4	31.5	31.6	29.3						
Milk lactose,	Early	43.4	41.9	42.4	42.9	0.58	*	ns	*	ns	*
g kg <sup>-1</sup>	Late	42.9	42.3	41.1	41.1						
Milk DM,	Early	116.5	123.5	103.9	107.1	2.13	*	***	***	ns	**
g kg <sup>-1</sup>	Late	109.5	116.8	106.1	107.2						
Milk urea	Early	10.7	7.1	10.9	9.0	0.29	***	***	***	ns	***
mmol L <sup>-1</sup>	Late	10.0	6.3	11.9	9.8						
Milk FFA,	Early	0.22	0.24	0.33	0.55	0.141	*	ns	*	ns	ns
mEq L <sup>-1</sup>	Late	0.23	0.19	0.29	0.17						

Table 1. Milk yield and constituents as affected by pasture type (P, C = cultivated pasture, R = forest rangeland), hay quality (H, HH = high quality, HL = Low quality) and grazing season (S) in dairy goats

SEM = standard error of the mean; ns – not significant (P > 0.05); \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001.

#### Conclusions

Goats grazing in a heterogeneous forest rangeland pasture had decreased milk yield and milk protein content compared with goats on cultivated pasture, but the milk quality was not impaired with respect to FFA. The effect of pasture type was similar in both Early and Late grazing seasons. Grazing resulted in milk with higher contents of fat, protein and DM, and lower contents of urea and FFA than hay diets. The results indicate that rangeland grazing does not necessarily lead to milk with a high content of FFA and to increased level of FFA during the grazing season.

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# References

Chilliard Y., Ferlay A., Rouel J. and Lamberet G. (2003) A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *Journal of Dairy Science* 86, 1751–1770.

Devold T.G., Nordbø R., Langsrud T., Svenning C., Jansen Brovold M., Sørensen E.S., Christensen B., Ådnøy T. and Vegarud G.E. (2011) Extreme frequencies of the  $\alpha_{s1}$  – casein "null" variant in milk from Norwegian dairy goats – implications for milk composition, micellar size and renneting properties. *Dairy Science and Technology* 91, 39–51.

Dønnem I., Eknæs M., and Randby Å.T. (2011a) Energy status, measured by computer tomography (CT)-scanning, and milk quality of dairy goats fed rations with various energy concentrations. *Livestock Science* 142, 235–244. Dønnem I., Randby Å.T. and Eknæs M. (2011b) Effect of grass silage harvesting time and level of concentrate supplementation on goat milk quality. *Animal Feed Science and Technology* 163, 118–129.

Eknæs M., Kolstad K., Volden H. and Hove K. (2006) Changes in body reserves and milk quality throughout lactation in dairy goats. *Small Ruminant Research* 63, 1–11.

Lachica M., Somlo R., Barroso F.G., Boza J. and Prieto C. (1999) Goats locomotion energy expenditure under range grazing conditions: seasonal variation. *Journal of Range Management* 52, 431–435.

Santini F.J., Lu C.D., Potchoiba M.J., Fernandez J.M. and Coleman S.W. (1992) Dietary fiber and milk yield, mastication, digestion, and rate of passage in goats fed alfalfa hay. *Journal of Dairy Science* 75, 209–219. SAS (2008) SAS User's Guide. Statistics Institute Inc., Cary, NC.

# Effect of perennial ryegrass cultivar on milk performance and dry matter intake in the spring-to-early-summer period

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# Abstract

The objective of this experiment was to examine the effect of perennial ryegrass cultivar (*Lolium perenne* L.) on milk yield and composition of grazing dairy cows. Four cultivars were sown as monocultures in 2009: Bealey and Astonenergy (tetraploid) and Abermagic and Spelga (diploid). The experiment was conducted from March to May 2011, with a 4×4 Latin square design with 7 cows per treatment. Animals were offered 16 kg herbage DM cow<sup>-1</sup> d<sup>-1</sup> and 1 kg concentrate cow<sup>-1</sup> d<sup>-1</sup>. There was no difference in the pre-grazing sward height among cultivars. Pre-grazing herbage mass was largest for Abermagic (P < 0.001; 1299 kg DM ha<sup>-1</sup>) and lowest on Bealey (1015 kg DM ha<sup>-1</sup>). Cultivar had a significant effect on post–grazing height (P < 0.05). The sheath height was highest for the diploids (P < 0.05; 6.3 cm) and lowest on the Astonenergy (5.4 cm). Astonenergy resulted in the largest milk production (P < 0.05; 32.0 kg cow<sup>-1</sup> d<sup>-1</sup>) compared with Abermagic and Spelga (30.1 kg cow<sup>-1</sup> d<sup>-1</sup>). Cultivar had a significant effect on milk protein percentage (P < 0.01) and milk solids yield (P < 0.01). Higher intake and milk production was achieved from the tetraploid swards compared with the diploids.

Keywords: milk production, animal performance, ploidy, Lolium perenne

# Introduction

Grass cultivar is of major importance due to its potential to influence animal production and sward productivity (Gowen *et al.*, 2003). Internationally, most cultivar-evaluation trials are conducted under cutting with little or no exposure to animal grazing. This provides little information on how a cultivar will influence the performance of grazing animals. The objective of this study was to examine the effects of 4 perennial ryegrass cultivars on the milk production and intake of spring-calving Holstein-Friesian dairy cows, in the spring to early summer period.

# Materials and methods

Four perennial ryegrass cultivars were sown in 2009. The cultivars included 2 tetraploids (Bealey, heading date 24 May; and Astonenergy, heading date 31 May) and 2 diploids (Spelga, heading date 24 May; and Abermagic, heading date 28 May). From calving, all animals were managed as one herd. In March 2011, 24 multiparous dairy cows were blocked into 6 groups of 4 cows. The groups were balanced for lactation number (mean 3.4; s.d. 0.21), calving date (mean 8 Feb, s.d. 3.1 days), milk yield (kg cow<sup>-1</sup> d<sup>-1</sup>), fat, protein and lactose content (g kg<sup>-1</sup>), bodyweight (kg) and body condition score. Cows within each group were then randomly assigned to a cultivar for a period of 14 days in a 4×4 Latin square design. The 14-day periods were split into a 9-day adaption period and a 5-day measurement period. Cows were offered 16 kg herbage DM cow<sup>-1</sup> d<sup>-1</sup> and 1 kg DM cow<sup>-1</sup> d<sup>-1</sup> concentrate. Fresh grass was offered daily. Herbage mass was measured twice weekly. Pre- and post-grazing sward height was measured daily. Extended tiller (ETH) and sheath height (SH) was measured on 100 tillers in each sward for 2 days, with measurement occurring in the same area for the post-grazed heights. Milk yield was measured daily and milk composition was measured at both morning and evening milking on days 1, 2 and 3 of the measurement period. Intake was measured using the n-alkane technique. Animal variables were analysed using a mixed model in SAS (SAS, 2008). Treatment and period were included in both the sward and milk performance analysis. Animal was classified as a random effect.

# Results

The results of the herbage measurements are presented in Table 1. Pre-grazing sward height was highest for Abermagic (9.1 cm; P = 0.09), but similar for the other 3 cultivars. Pregrazing herbage mass was highest (P < 0.001) for Abermagic (1299 kg DM ha<sup>-1</sup>), intermediate for Astonenergy and Spelga (1062 kg DM ha<sup>-1</sup>) and lowest for Bealey (1015 kg DM ha<sup>-1</sup>). There was no difference in pre-grazing ETH among cultivars. Pre-grazing SH was highest (P < 0.05) for Spelga and Abermagic, and lowest for Astonenergy. Post–grazing sward height was highest for Spelga (4.1 cm; P < 0.05) compared with the other cultivars that were similar. Post–grazing ETH was highest for Spelga (5.2 cm); this was not significantly different to the Bealey or Astonenergy (4.5 cm). Post-grazing SH was lowest for Astonenergy (4.7 cm; P < 0.01) and highest for Spelga (5.6 cm).

Table 1	. Pre and post	grazing herbage	measurements	for 4 cultiva	ars of pe	erennial r	yegrass o	during th	ıe
spring t	o early summe	er period							

	Bealey	Astonenergy	Spelga	Abermagic	SE	P-value
Pre-grazing sward height (cm)	8.4ª	8.4ª	$8.8^{ab}$	9.1 <sup>b</sup>	0.2	0.09
Pre-grazing herbage mass (kg DM ha <sup>-1</sup> )	1015 <sup>a</sup>	1099 <sup>b</sup>	1142 <sup>b</sup>	1299°	28	0.001
Pre-grazing extended tiller height (cm)	20.4	22.9	19.9	20.1	1.6	ns
Pre-grazing sheath height (cm)	5.7 <sup>ac</sup>	5.4ª	6.4 <sup>b</sup>	6.2 <sup>bc</sup>	0.3	0.05
Sward density (kg of DM cm <sup>-1</sup> ha <sup>-1</sup> )	208 <sup>a</sup>	246 <sup>b</sup>	285°	327 <sup>d</sup>	10	0.001
Area $(m^2 \text{ per cow } d^{-1})$	193ª	153 <sup>b</sup>	150 <sup>bc</sup>	123°	10	0.001
Post-grazing sward height (cm)	3.7ª	3.9 <sup>ab</sup>	4.1 <sup>b</sup>	3.9 <sup>ab</sup>	0.1	0.05
Post-grazing extended tiller height (cm)	4.5 <sup>a</sup>	4.6ª	5.2ª	4.1 <sup>ab</sup>	0.3	0.09
Post-grazing sheath height (cm)	5.1ª	4.7 <sup>b</sup>	5.6°	5.2 <sup>ac</sup>	0.2	0.01

SE = standard error.

<sup>abc</sup> Means within a row with different superscripts differ significantly (P < 0.05).

The results of the milk yield, composition and DM intake from the study are presented in Table 2. Milk yield was highest (P < 0.05) for Astonenergy (31.9 kg cow<sup>-1</sup> day<sup>-1</sup>), intermediate for Bealey (31.3 kg cow<sup>-1</sup> d<sup>-1</sup>) and lowest for Spelga and Abermagic (30.1 kg cow<sup>-1</sup> day<sup>-1</sup>). Milk protein content was smallest (P < 0.01) for Spelga (3.3 g kg<sup>-1</sup>) compared to the other cultivars (3.4 g kg<sup>-1</sup>). Milk solids yield was similar for Bealey, Astonenergy and Abermagic (2.4 kg cow<sup>-1</sup> d<sup>-1</sup>) and lower (P < 0.01) for Spelga (2.3 kg cow<sup>-1</sup> d<sup>-1</sup>). The free leaf lamina (FLL) available to each group in the pre-grazed sward was 14.7, 17.5, 13.5 and 13.9 cm for the Bealey, Astonenergy, Spelga and Abermagic treatments respectively.

#### Discussion

The results of this study show that cultivar can significantly influence the performance of grazing dairy cows. Pre-grazing sheath height appears to have influenced the post-grazing height, with higher sheath heights in the pre-grazing swards possibly limiting the ability of the cows to graze deep into the horizon. It appears that sheath height had a greater influence on the ability of the animals to graze into the sward than tiller height. The difference between the sheath height and the tiller height determines the FLL, or the amount of leaf that is

available to the grazing animal. The FLL available was greater for the Astonenergy treatments, resulting in a greater proportion of a higher quality feed (leaf) in the animals' diet. As animals were offered a daily allowance of 16 kg DM cow<sup>-1</sup> d<sup>-1</sup>, differences in pre–grazing herbage mass influenced the actual area which was offered per day. As herbage mass increased, the area offered per day decreased. Within the study, swards were managed in order to ensure that herbage mass did not exceed 1600 kg DM ha<sup>-1</sup>. The differences in area offered, FLL and available leaf explain the greater intake achieved on the Astonenergy swards, and, as these animals were also offered the greatest proportion of leaf, they achieved the highest milk yield, closely followed by the Bealey treatment. The higher intakes and milk production on tetraploids compared with diploids is similar to the findings Hageman *et al.* (1993).

of 4 cultivars of perchin	r + cuttvars of perennial ryegrass in the spring period								
	Bealey	Astonenergy	Spelga	Abermagic	SE	P-value			
Milk yield (kg d <sup>-1</sup> )	31.4 <sup>ab</sup>	32.0ª	30.2 <sup>b</sup>	30.1 <sup>b</sup>	0.8	0.05			
Milk fat (g kg <sup>-1</sup> )	4.6	4.3	4.3	4.6	0.1	0.07			
Milk protein (g kg <sup>-1</sup> )	3.4ª	3.4ª	3.3 <sup>b</sup>	3.4ª	0.04	0.01			
Milk lactose (g kg <sup>-1</sup> )	4.7	4.7	4.7	4.7	0.03	ns			
Milk solids (kg d <sup>-1</sup> )	2.5ª	2.4ª	2.3 <sup>b</sup>	2.4ª	0.1	0.01			

16.4<sup>a</sup>

16.1ª

0.05

0.4

Table 2. Milk production, composition and dry matter intake results of lactating cows grazing on one of 4 cultivars of perennial ryegrass in the spring period

<sup>abc</sup> Means within a row with different superscripts differ significantly (P < 0.05).

17.5<sup>b</sup>

16.1ª

#### Conclusions

Intake (kg DM d<sup>-1</sup>)

The results of this study indicate that in the spring-to-early-summer period, cows offered tetraploid swards with a high leaf content and of intermediate herbage mass (1100 kg DM ha<sup>-1</sup>), have the potential to achieve higher levels of dry matter intake and, as a result, higher milk yield output compared with cows offered diploid cultivars. Despite the higher intake and milk yield, there were only small differences in milk solids yield per cow per day, indicating that the allowance may have been restrictive; however, chemical composition results may explain the differences in more detail.

#### Acknowledgements

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#### References

Gowen N., O'Donovan M., Casey I., Rath M., Delaby L. and Stakelum G. (2003) The effect of grass cultivars differing in heading date and ploidy on the performance and dry matter intake of spring calving dairy cows at pasture. *Animal Research* 52, 321–336.

Hageman I.W., Lantinga E.A., Schlepers H. and Neuteboom J.H. (1993) Herbage intake, digestibility characteristics and milk production of a diploid and tow tetraploid cultivars of perennial ryegrass. In: *Proceedings for the XVII International Grassland Congress*, Palmerston North, New Zealand, pp. 459–460. SAS. 2008. SAS User's Guide. Statistics Institute Inc., Cary, NC.

# Quality of *Montasio* cheese from Italian Simmental cows grazing on mountain pasture or reared indoors

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# Abstract

The aim of this study was to compare *Montasio* cheese made from milk of 120 Italian Simmental cows reared under two different systems. On an alpine farm, 60 cows were maintained on *Gramineae*-rich pasture and supplemented with 2 kg head day<sup>-1</sup> of concentrate on average. The other 60 cows were kept indoors and fed a hay-based diet. Cheese production was repeated in two periods (early July and late August) and processed for each period for 3 consecutive days according to traditional production technology of PDO *Montasio*. Analyses of chemical, textural and fatty acids were performed on two-month-ripened cheeses. Pasture-derived cheese was significantly different from hay-derived cheese in terms of chemical composition (higher dry matter and fat, lower protein), colour (more yellow-red) and textural properties (higher hardness, gumminess and chewiness). In addition, it showed a fatty acids profile more beneficial for human health.

Keywords: cheese quality, Italian Simmental cows, mountain pasture, indoor housing

# Introduction

The added value of mountain cheese is linked to the ability to evoke the production area and its environmental, historical and cultural values as well as its objective nutritional and sensorial characteristics (Bovolenta *et al.*, 2011). For instance, *Montasio* cheese is one of the most important PDO (Protected Designation of Origin) and traditional products in North-East Italy. However, although it takes its name from a mountain plateau, it is produced and manufactured largely in lowlands. Recently, in order to valorize the mountain cheese and to link it to the breed more present in this area, the production of *Montasio* PDO was combined with two additional labels: 'Mountain Product' (*PDM* in Italy) and 'only Italian Simmental breed' (*Solo di Pezzata Rossa Italiana* in Italy). The aim of the study was to compare the quality properties of this cheese produced in two periods from milk of cows grazing on mountain pasture or fed indoors with a hay-based diet.

# Materials and methods

The trial was carried out using 120 lactating Italian Simmental cows: 60 grazing on *Gramineae*rich mountain pasture (*Malga Montasio*, Udine, Italy; 46°24'45" N, 13°25'53" E; 1500–1800 m asl) and supplemented with 2 kg head day<sup>-1</sup> of concentrate; and 60 were maintained indoors and fed with local hay and concentrates (ratio 60:40). Cheese production was repeated in early July and late August. For each period, a mixture of evening and morning milk was processed for 3 consecutive days according to the rules of PDO *Montasio*. Two cheeses for each cheese-making were ripened for 2 months in a ripening cellar with controlled temperature (12°C) and humidity (85%). The experimental cheeses were analysed for dry matter (DM), fat, total nitrogen (TN) and soluble nitrogen at pH 4.6 (SN). The ripening index (RI) was calculated as ratio between SN and TN. A spectrophotometer (Minolta CM2600d) was used for measuring colorimetric parameters (L\*, a\*, b\*). Texture profile analysis (TPA; TA plus, Lloyd Instruments) was carried out on samples cylinders (20 mm  $\times$  20 mm; deformation: 50%; speed: 100 mm min<sup>-1</sup>). Fatty acids (FA) methyl esters were determined by GC system (5300 Carlo Erba, SP-2380 column) after lipid extraction. Data were analysed using the GLM repeated measures procedure with period as within-subject factor and rearing systems as between-subject factor (SPSS v. 17, SPSS Inc., Illinois).

#### **Results and discussion**

Pasture-derived cheese showed significantly higher DM and fat, but lower protein content compared with hay-derived cheese. The effect of period was limited to DM, higher at August than at July (Table 1). It is well known that high dietary energy levels reduce the milk fat concentration by reducing the synthesis of acetic acid in the rumen (Arriaga-Jordan and Holmes, 1986). Moreover, the lower protein concentration in Pasture-derived cheese could be due to the low energy supply and hypoxia, which characterize the dairy cows grazing on high mountain pasture (Leiber et al., 2006). This cheese showed lower RI and pH. The RI is used to assess proteolysis in cheese by considering the hydrolysis of casein. It depends on many factors such as protease content, its activity in milk and processing conditions. Both cheeses were produced with the same technological process, but probably they were characterized by a different raw milk microbial flora. Pasture-derived cheese was significantly more red (a index +1.5) and yellow (b index +8.9) than hay-derived cheese. Moreover, cheese produced in August had higher value of redness than that produced in July. The more yellow-red coloration depends on high carotenoid content in grass, which can change with the phenological stage of the pasture plants (Coppa et al., 2011). The TPA parameters hardness, gumminess and chewiness were significantly higher in the pasture-derived cheese and in cheese produced in August (Table 2). Texture depends on a complex interaction between chemical composition and ripening parameters of cheese. The different humidity and RI could have induced these differences with particular regard to hardness (Gunasekaran and Ak, 2003). Concerning the FA profile (Table 3), interaction was always ordinal from the perspective of rearing systems and non-ordinal from the perspective of period. Pasture-derived cheese had lower levels of short and medium chain saturated FA (SFA), with the exception of C4:0. The effect of period was limited to C4:0 and SFA, higher in August than in July. These SFA originate by mammary de novo synthesis, which is favoured by increasing the availability of ruminal volatile FA linked to high level of dietary concentrate (Bargo et al., 2006). Pasture-derived cheese was richer in C18:1, C18:3 n-3 and conjugated linoleic acid (CLA) and consequently showed higher values of mono-unsaturated FA and polyunsaturated FA in agreement with Coppa et al. (2011). Indeed, with respect to total FA, grass is comprised mainly of C18:3 n-3, and from its biohydrogenation products the mammary gland is able to synthesise CLA (Chillard et al., 2007).

	Rearing system (R)		Peri	od (P)	SEM	Significance(1)		
	Pasture	Indoor	July	August	SEIM	R	Р	RxP
Dry matter (DM), g 100g <sup>-1</sup>	67.6	65.6	65.8	67.5	0.20	**	*	ns
Fat, g 100g <sup>-1</sup> DM	54.2	51.0	52.4	52.8	0.23	**	ns	ns
Protein, g 100g <sup>-1</sup> DM	39.1	42.3	41.2	40.1	0.18	**	ns	ns
Ripening index (RI)(2)	10.9	13.4	11.5	12.8	0.32	*	ns	ns
pH	5.27	5.37	5.33	5.31	0.010	**	ns	ns
$L^*$ (lightness)	76.3	78.5	76.7	78.0	0.40	ns	ns	ns
$a^*$ (redness)	2.1	0.6	1.1	1.6	0.04	**	**	ns
<i>b</i> * (yellowness)	24.2	15.3	19.5	19.9	0.22	**	ns	ns

Table 1. Chemical composition, acidity and colorimetric parameters of cheese

(1) \* P < 0.05; \*\* P < 0.01; ns – P > 0.05. (2) ratio (\*100) between Soluble Nitrogen and Total Nitrogen.

*	5								
	Rearing system (R)		Perio	od (P)	SEM	Significance(1)			
	Pasture	Indoor	July	August	SEIVI	R	Р	RxP	
Hardness, N	70.8	54.8	58.2	67.4	1.30	**	*	ns	
Cohesiveness, g 100g <sup>-1</sup>	57.0	58.2	57.7	57.6	0.37	ns	ns	ns	
Springiness, g 100g <sup>-1</sup>	81.4	81.8	81.9	81.3	0.23	ns	ns	ns	
Adhesiveness, J	0.9	0.9	0.7	1.1	0.04	ns	ns	ns	
Gumminess, N	40.4	31.8	33.4	38.8	0.76	**	*	ns	
Chewiness, N	32.8	26.1	27.4	31.5	0.57	**	*	ns	
		0.05							

Table 2. Texture profile analysis of cheese

(1) \* P < 0.05; \*\* P < 0.01; ns -P > 0.05.

Table 3. Fatty acids composition (g 100g-1 fatty acids methyl esters) of cheese

	Rearing		Peric	Period (P)		Significance(1		re(1)
	system (R)		I UIII					
	Pasture	Indoor	July	August		R	Р	RxP
C4:0	3.20	2.97	3.00	3.17	0.049	ns	*	ns
C10:0	2.72	3.22	2.97	2.97	0.015	**	ns	*
C12:0	3.18	3.89	3.53	3.54	0.011	**	ns	*
C14:0	11.41	13.00	12.19	12.22	0.038	**	ns	**
C16:0	28.31	33.53	30.71	31.14	0.059	**	ns	ns
C18:0	10.90	10.21	10.61	10.50	0.068	**	ns	ns
C18:1 n-9 trans	6.10	2.83	4.36	4.57	0.140	**	ns	ns
C18:1 n-9 cis	21.88	20.66	21.90	20.63	0.138	*	ns	ns
C18:3 n-3	1.15	0.44	0.76	0.83	0.017	**	*	**
CLA (conjugated linoleic acid)(2)	1.54	0.48	0.91	1.11	0.015	**	**	**
SFA (saturated fatty acids)	65.16	72.13	68.33	68.95	0.136	**	*	ns
MUFA (monounsaturated fatty acids)	31.28	26.73	29.49	28.53	0.133	**	**	**
PUFA (polyunsaturated fatty acids)	3.56	1.15	2.18	2.52	0.032	**	**	**

(1) \* P < 0.05; \*\* P < 0.01; ns -P > 0.05; (2) sum of *trans* 10 *cis* 12 and *cis* 9 *trans* 11 isomers.

#### Conclusions

Difference between cheeses can be ascribed mainly to the animal rearing system rather than the period of production. Pasture-derived cheese was different in terms of chemical composition, colour and textural properties, and it showed a fatty acids profile that was more beneficial for human health. These positive characteristics, if well communicated to consumers, can be useful for the valorisation of pasture-derived products.

#### References

Arriaga-Jordan C.M. and Holmes W. (1986) The effect of cereal concentrate supplementation on the digestibility of herbage-based diets for lactating dairy cows. *Journal of Agricultural Science* 106, 581–592. Bargo F., Delahoy J.E., Schroeder G.F. and Muller L.D. (2006) Milk fatty acid composition of dairy cows grazing at two pasture allowances and supplemented with different levels and sources of concentrate. *Animal Feed Science and Technology* 125, 17–31.

Bovolenta S., Dovier S. and Parente G. (2011) Dairy production systems in the Italian alpine area. In: ACW and ITEP (eds) *Contribution of mountain pastures to agriculture and environment*. The 16th Meeting FAO CIHEAM Mountain Pastures Network. Kraków, Poland, pp. 143–146.

Chilliard Y., Glasser F., Ferlay A., Bernard L., Rouel J. and Doreau M. (2007) Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science and Technology* 109, 828–855. Coppa M., Ferlay A., Monsallier F., Verdier-Metz I., Pradel P., Didienne R., Farruggia A., Montel M.C. and Martin B. (2011) Milk fatty acid composition and cheese texture and appearance from cows fed hay or different grazing systems on upland pastures. *Journal of Dairy Science* 94, 1132–1145.

Gunasekaran S. and Ak M.M. (2003) *Cheese rheology and texture*. CRC Press, Florida, USA, 437 pp. Leiber F., Kreuzer M., Leuenberger H. and Wettstein H.R. (2006) Contribution of diet type and pasture conditions to the influence of high altitude grazing on intake, performance and composition and renneting properties of the milk of cows. *Animal Research* 55, 37–53.

# Effect of gender on lamb meat quality

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# Abstract

In Norway, most lambs are slaughtered at the end of the grazing season in September. An increased demand for fresh meat during the off-season may change this pattern. Castration of male lambs is not permitted, and off-season slaughtering may affect the acceptability of the meat. The objective of this study was to determine the effect of gender on meat quality from Norwegian White Sheep lambs slaughtered in September at the age of five months. Twentynine male lambs and 46 female lambs were included in the experiment. Loin samples of *M*. *Longissimus dorsi* were analysed for sensory profile. Meat from male lambs had higher scores for the less pleasant sensory attributes of cloying (P < 0.05) and rancid flavour (P = 0.08), and lower scores for the more pleasant attributes of sour and sweet taste (P < 0.05 for both attributes) compared with meat from female lambs. The differences were more apparent between lambs grazing ryegrass than between lambs fed a concentrate and roughage diet. It is concluded that even at the normal slaughtering time in September, significant differences between genders may occur.

Keywords: concentrate, grass silage, *Lolium multiflorum*, Norwegian White Sheep, sensory profile

# Introduction

In Norway, lambs traditionally graze with their dams on unimproved mountain pastures during the summer. Most lambs are slaughtered directly off the pasture in the autumn at the age of four to five months. However, due to variations in live weight, lambs are sometimes fattened on cultivated pastures or supplemented with concentrates to increase their carcass weight before slaughtering. An increased demand for fresh meat during the off-season may change this pattern. Castration of male lambs is not permitted, and off-season slaughtering may affect the acceptability of the meat. Studies have reported variation in the quality (sensory profile and fatty acid composition) of lamb meat due to several factors, with diet and energy availability being most prominent (e.g. Aurousseau et al., 2007). Norwegian sheep are known to be seasonal breeders with females mainly in heat in November and December. During this period, male lambs are seldom slaughtered due to sexual maturation that causes unpleasant meat characteristics. Mushi et al. (2008) found significant off-flavours in meat from male lambs compared with that of female lambs when slaughtering took place before the mating season in October/November. Even when lambs were slaughtered in mid-September, minor differences between genders were still found. However, these results are based only on one study. The objective of this study was to test the hypothesis that male lambs slaughtered at the age of five months produced meat with a similar sensory profile to that of female lambs.

### Materials and methods

The experiment was carried out at an experimental farm at Tjøtta in Northern Norway (65°49 N, 12°25 E) from May to September 2008. The effect of gender on meat quality was compared between a group of suckling male and female lambs slaughtered directly from a semi-natural pasture and lambs of both genders that were grazing the semi-natural pasture until August. The lambs were then weaned and subjected to either stall-feeding with concentrate and grass silage or grazing on Italian ryegrass (*Lolium multiflorum*). One-hundred and fifty Norwegian White Sheep lambs were born indoors during April and May and turned out to graze a semi-natural pasture with their dams in mid-May. A total of 40 lambs were selected for the stall-feeding treatment and another 40 lambs were grazing ryegrass until slaughter in September at the age of five months.

One-hundred lambs from the different treatments were sent to the local abattoir where they were shorn and slaughtered immediately after arrival. After slaughter, a total of 75 carcasses (29 males and 46 females) were selected for descriptive sensory analyses. After chilling, the saddles (*M. longissimus dorsi*) were removed from the carcasses, wrapped and vacuumpacked in sealable polyamide bags at the abattoir and brought to Nofima Mat for sensory analysis. The saddles were aged for six days. Meat samples were then heated in a 70°C water bath for 40 min and served from a hot plate at 65°C to a sensory panel consisting of nine trained, selected assessors at Nofima Mat (ISO, 1993). The nine assessors developed a test vocabulary to describe the differences between samples and they agreed upon a list of 19 attributes in total. A continuous, non-structural scale from 1 (lowest intensity) to 9 (highest intensity) was used in the evaluation (ISO, 1999). (For more details see Lind *et al.*, 2011). Sensory data were analysed using a mixed-model ANOVA (PROC MIXED, SAS for Windows) with the assessor and animal (nested within gender) effects and their interaction as random effects, and gender as fixed effect.

#### **Results and discussion**

Table 1 shows average values for some of the sensory attributes and results from ANOVA comparing results for male and female lambs in the experiment. Meat from female lambs has a higher intensity in sweetness (P < 0.05), sourcess (P < 0.05) and a lower intensity in cloying flavour (P < 0.05) than meat from male lambs. In addition, Table 1 shows a corresponding tendency for sweet odour (P = 0.08) and sour odour (P = 0.07) i.e. higher intensity for female than male. The table also shows a tendency to a higher intensity in gamey and rancid flavour (both P = 0.08) and lower intensity in hardness (P = 0.07) in meat from male lambs than from female lambs. Flavour intensity seems to be the sensory attribute most often influenced by gender (Arsenos et al., 2002). In the present study flavour intensity was not included as a separate attribute. Instead this may be seen as the sum of other flavour attributes included: i.e. higher intensities in metallic, cloying, gamey and rancid may be interpreted as a higher intensity in flavour. Sweet odour and taste are traits often considered to be positive in meat. Results from this experiment show that meat from female lambs has a higher intensity for these attributes. This is in line with findings of Mushi et al. (2008) who demonstrated that meat from female lambs slaughtered at the age of six months had a higher intensity in sour taste compared with meat from male lambs slaughtered at the same age. The differences in the present experiment were more apparent between lambs grazing ryegrass than between lambs fed concentrate and roughage. The higher intensity in cloying flavour in meat from male lambs found in this experiment is also in agreement with Mushi et al. (2008). When meat from male lambs scored higher in cloving taste, ram flavour was also found to be more intense compared to meat from female lambs. This suggests that cloying flavour is linked to sexual maturation of male lambs.

Attribute	Male	Female	SEM	Р
Odour				
Sweet	3.31	3.47	0.9	**
Sour	3.20	3.50	1.1	**
Rancid	1.67	1.43	1.1	ns
Taste				
Sweet	3.12	3.32	0.8	*
Sour	3.08	3.48	1.1	*
Flavour				
Cloying	3.06	2.80	1.6	*
Gamey	2.18	2.36	1.1	**
Rancid	1.95	1.60	1.4	**
Texture				
Hardness	4.30	4.05	1.1	**
Tenderness	5.67	5.89	1.4	ns
Fattiness	4.77	4.77	0.9	ns
Juiciness	5.43	5.55	1.0	**

Table 1. Least square means of sensory profiles of meat from male and female lambs (evaluated on a scale of 1 to 9)

SEM – Standard Error Mean; ns – not significant; \*P < 0.05, \*\*0.05 < P < 0.1.

#### Conclusions

The results indicate that meat from female lambs should be prioritised over meat from male lambs for production of fresh quality products at all seasons, whilst meat from male lambs could be used for less taste-sensitive dishes like dried and salted meat also high in demand. Off-season fresh meat should preferably be produced from female lambs. Furthermore, male lambs should be given optimum conditions to be ready for slaughter early in the season thereby reducing the risk of unwanted sensory characteristics in the meat.

#### References

Arsenos G., Banos G., Fortomaris P., Katsaounis N., Stamataris C., Tsaras L. and Zygoyiannis D. (2002) Eating quality of lamb meat: effect of breed, sex, degree of maturity and nutritional management. *Meat Science* 60, 379–387.

Aurousseau B., Bauchart D., Faure X., Galot A.L., Prache S., Micol D. and Priolo A. (2007) Indoor fattening of lambs raised on pasture: (1) Influence of the stall finishing duration on lipid classes and fatty acids in the *longissimus thoracis* muscle. *Meat Science* 76, 241–252.

ISO (1993) Sensory Analysis-methodology-General Guidance for the selection, training and monitoring of assessors – part 1 Selected assessors. ISO 8586, Geneva, Switzerland: International Organization for Standardization.

ISO (1999) Sensory analysis, vocabulary. Norsk standard NS-ISO 5492.

Lind V., Berg J., Eilertsen S.M., Hersleth M. and Eik L.O. (2011) Effect of gender on meat quality in lamb from extensive and intensive grazing systems when slaughtered at the end of the growing system. *Meat Science* 88, 305–310.

Mushi D.E., Eik L.O., Sørheim O., Ådnøy T. and Haugen J.E. (2008) Effect of sex of animals and time of slaughter on sensory quality of meat from Norwegian lambs. *Acta Agriculturae Scandinavia Section A* 58, 31–36.

# New developments in the Netherlands: dairies reward grazing because of public perception

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# Abstract

Changing societal drivers and consumer demands require systems that provide desired human foods produced through sustainable production systems. Milk composition has changed with higher milk-solid contents and lower unsaturated fatty acid concentrations. The latter can be modified on-farm by feeding system and grazing management, but the trend in the Netherlands is for cows to be indoors more and to consume less fresh grass. Although research has provided data showing beneficial effects of fresh herbage in the dairy production chain, it is not facts but sentiments and marketing that have dictated the political agenda. Action groups have triggered the public debate, mainly from the viewpoint of animal welfare. This has provoked much discussion and raised concerns in public opinion and in politics. In 2011 the major Dutch dairy company changed its policy in favour of promoting grazing, mainly to preserve the natural image and for providing dairy farmers with a societal license to produce.

Keywords: grass-fed milk, grazing, dairy chain, consumers, retail, public opinion, market

### Introduction

Changing societal drivers (e.g., landscape values, animal welfare) and consumer demands (e.g., tasty/healthy products) require systems that provide desired human foods produced through sustainable production processes. High-fat human diets, especially those rich in saturated fats, are often claimed to have detrimental effects on cardiovascular disease-risk factors such as blood low-density lipoprotein cholesterol. Dairy products contribute 15-20% of human intake of total fat, 25–33% of saturated fat and about 15% of dietary cholesterol. During the last few decades, the composition of milk has changed due to animal breeding, cow diet and farm management. In the Netherlands, the milk production per cow greatly increased, as have the fat and protein contents. In many countries the fatty acid (FA) composition of milk has become less favourable from a human nutrition viewpoint, as unsaturated FA concentrations have declined; e.g. in the 1960s, during summer the Dutch farm milk contained, on average, 15 g kg<sup>-1</sup> conjugated linoleic acid (CLA), but in 2001–2002 the average was 7 g kg<sup>-1</sup> between June and August, and 5 g kg<sup>-1</sup> in spring and autumn (Elgersma et al., 2006a; b). This is due to an increased demand for energy in cows, and changed feeding and management practices, notably higher proportions of concentrates and silages in diets with less grazing. The trend for cows to be kept more indoors has raised public concern. Economic analyses have shown benefits of grazing, as conserved feed is more expensive than fresh herbage. For similar climatic conditions, grazing is more economically attractive than indoor feeding systems (Peyraud et al., 2010). The more grass that cows eat at pasture, the larger is the farmer's income (Van den Pol-van Dasselaar et al., 2010). However, cows with very high milk yield potential cannot meet their energy requirements from grass alone, partly due to insufficient intake. Another bottleneck with very large herds often is the distance of paddocks to the milking parlour, particularly with farms using a milking robot. Grasslands, however, could offer considerable scope to help create dairy product differentiation in increasingly competitive markets. The aim of our studies was to raise awareness for regional and temporal changes in milk FA composition in relation to cow diet and hours at pasture.

#### Materials and methods

Pooled farm tank milk samples were analysed monthly and concentrations of CLA were presented separately for four regions of the Netherlands. In a second study, CLA concentrations were measured in milk samples between individual farmers. Questionnaires provided associated information on soil type, feeding system and hours that cows spent at pasture. Temporal and regional patterns were analysed.

### **Results and discussion**

CLA concentrations in pooled tank milk from the West and North of the Netherlands, where cows are fed mainly grass, and soils consist mainly of clay and peat, were higher than in the South and East with prevailing sandy soils, where much maize is cultivated and fed as silage, whereas less grazing is practised. Regional differences were striking, particularly in the grass growing season (mid April – late October) (Figure 1).



Figure 1. Concentrations of conjugated linoleic acid (CLA) in pooled farm tank milk of 4 regions (West, North, East and South) in the Netherlands from August 2001 till July 2002

Large variation in milk CLA was found among farmers; however, they were generally clearly related to feeding system: farmers with summer feeding (i.e. year-round silage and concentrates without fresh grass) had the lowest and farmers whose cows had day and night access to pasture had the highest CLA concentrations in their milk. Restricted stocking (i.e. daytime grazing and indoors at night) resulted in intermediate levels (Figure 2).

Milk from cows fed fresh green forage, especially in grazing systems, has much higher unsaturated FA concentrations than milk from silage-fed cows (Elgersma *et al.*, 2006b). Therefore, in farming systems where fresh herbage is fed, CLA levels are on average lower during the indoor season than during the grass-growing season (Figure 2).



Figure 2. Concentrations of conjugated linoleic acid (CLA) in farm tank milk in February (indoor situation) and September (grazing season) of Dutch farms with different feeding management

Our results were picked up by the media (TV, interviews) in 2004 and communicated to the dairy industry. In 2007, one major dairy cooperative started to pay a modest premium price ( $\notin 0.05$  per 100 kg milk) to farmers who had their cows on pasture and launched a product 'grass-fed milk', while the other launched a special brand made from milk produced by selected farmers who fed their cows a special concentrate (Nutex), based on linseed. However, it proved difficult to maintain a constant level of FA concentrations during the year and this special brand was withdrawn in 2011 by the meanwhile-merged cooperative, FrieslandCampina.

Although research showed benefits of grazing in terms of improving milk FA composition (Elgersma et al., 2006a; b) and economic (Van den Pol-van Dasselaar et al., 2010) as well as ecological farm performance (Pevraud et al., 2010), it is not facts but sentiments that have dictated the political agenda. The Dutch consider cows on grassland as their cultural heritage and associate grazing with landscape values, naturalness, and being good for the animals. Whereas pigs and poultry are kept indoors and are associated with bio-industry and odours, cows have a positive image. The fact that more cows are kept indoors was highlighted by action groups (e.g., Wakker Dier), who triggered the public debate, mainly from the viewpoint of animal welfare. This has provoked much discussion and raised concern in public opinion and in politics. Retailers changed their policy and decided to replace their private label dairy products with products based on milk from grass-fed cows. In September 2011, the FrieslandCampina dairy company changed its policy in favour of promoting grazing, mainly to preserve the natural image and for providing dairy farmers a societal license to produce. From 2012 onward they will pay a ten-fold higher premium price (€ 0.50 per 100 kg) only to farmers who have their cows on pasture for at least 120 days year<sup>-1</sup> for minimal 6 h day<sup>-1</sup>. In this way, primary producers who practise grazing can benefit from the higher market value at the end of the production chain.

#### Conclusions

On the basis of research data the importance of fresh grass in the diet was demonstrated for enhancing naturally and economically the concentrations of CLA and unsaturated fatty acids in milk. We showed the seasonal variation in contrasting regions, in association with feeding system. Milk composition can be modified on-farm by feeding system and grazing management, but grass availability differs across regions and soil types. Results have been used by action groups to trigger the debate; they provoked much discussion also on animal welfare and this raised concern in public opinion and in politics. Besides facts, sentiments in the media dictated the political agenda. Retail played a very important role. In 2011, the major Dutch dairy cooperative changed its policy in favour of promoting and rewarding grazing.

#### References

Elgersma A., Dijkstra J. and Tamminga S. (2006a) (eds) Fresh herbage for dairy cattle: the key to a sustainable food chain. *Wageningen UR Frontis Series 18. Springer-Verlag*.

Elgersma A., Tamminga S. and Ellen G. (2006b) Modifying milk composition through forage. *Animal Feed Science and Technology* 131, 207–225.

Peyraud J.L., Pol-van Dasselaar A. van den, Dillon P. and Delaby L. (2010) Producing milk from grazing to reconcile economic and environmental performances. *Grassland Science in Europe* 15, 865–879.

Van den Pol-van Dasselaar A., Haan M. de and Philipsen A.P. (2010) Simulation of the effect of grass intake on the farmers's income. *Grassland Science in Europe* 15, 100–102.

# Session 3

# Non-fodder use of grassland

# Perspectives of energy production from grassland biomass for atmospheric greenhouse gas mitigation

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## Abstract

Grassland ecosystems may act as a source or a sink of greenhouse gases (GHG), depending on their management as well as on soil, climate and atmospheric  $CO_2$  factors. The conversion of part of these grasslands to bioenergy production systems would have impacts on their GHG balance, but further experiments on the carbon and GHG budgets of grasslands shifted from livestock to bioenergy production are required. While improved biogas production from residual biomasses gives net GHG savings, this technique is frequently used to convert intensively managed grasslands, displacing food production elsewhere. Combustion gives higher conversion efficiencies especially with semi-natural grassland, a resource which remains increasingly unused. Mechanical separation of grassland silage improves fuel quality and increases energetic conversion efficiency. Thus, such a use would not cause a displacement of food production leading to less socio-economic effects and superior GHG savings.

Keywords: grassland biomass, bioenergy, greenhouse gas mitigation, hay combustion, anaerobic digestion, Integrated Generation of Solid Fuel and Biogas from Biomass

## Introduction

Energy from biomass is a contemporary issue in science and politics. The growing demand for energy worldwide, as well as the limited and unstable supply of fossil fuels, is responsible for increasing energy prices. Furthermore, constantly increasing  $CO_2$  emissions are intensifying climate change. Thus, both from an economic and an ecological point of view, there is an urgent need to develop sustainable, eco-friendly energy supplies.

Grassland plays an important role as part of the world's biomass potential, since 26% of the total land area and 68% of the agricultural area is covered by permanent pastures and meadows. In many European countries, the traditional management of large areas of permanent grassland as a feed source in animal husbandry is at risk of being abandoned, because the herbage does not meet the yield and quality demands of intensive livestock production (Isselstein *et al.*, 2005). Furthermore, grassland is increasingly converted to arable land where soil conditions allow this, although recent European regulations seek to prevent this (Anonymous, 2009). There is increasing evidence that the conversion is mainly in favour of maize, grown for fodder and, in some countries, for bioenergy production.

Semi-natural grasslands are often hot spots of biodiversity, and host many specialized plants, animals and microbes, all nested in a great variety of microhabitats (Mariott *et al.*, 2004; Clergue *et al.*, 2005). Consequently in Europe, some of the most critical nature conservation issues today relate to changes from traditional to modern farming practices on habitats, which are used for grazing and, in some other areas, where intensification is not possible or is uneconomic, resulting in the abandonment of farmland. Grassland soils are large stores

of carbon (C) and thus can act as a net sink for atmospheric  $CO_2$  (i.e. C sequestration). Nitrous oxide (N<sub>2</sub>O) is emitted by fertilized grassland soils and animal waste storage systems (Freibauer *et al.*, 2004). Enteric fermentation produces methane (CH<sub>4</sub>), which is emitted by livestock at grazing and can be exchanged with the soil (Soussana *et al.*, 2004). Therefore, grasslands contribute to the biosphere–atmosphere exchange of radiatively active trace gases, with their fluxes intimately linked to management.

This paper reviews the contribution of grassland ecosystems for GHG mitigation and describes the status of the utilization of grassland biomass for energy purposes by focussing firstly on the two most common conversion processes, i.e. biogas production from grass silage and combustion of hay. As both techniques face important limitations especially with regard to low-input high-diversity (LIHD) grassland, the strengths and weaknesses of an innovative concept, i.e. the "Integrated Generation of Solid Fuel and Biogas from Biomass" is discussed.

#### The role of grassland for atmospheric greenhouse gas mitigation

Of the three greenhouse gases that are exchanged by grasslands, CO, is exchanged with the soil and vegetation, N<sub>2</sub>O is emitted by soils and CH<sub>4</sub> is emitted by livestock at grazing and can be exchanged with the soil. The magnitude of these greenhouse gas exchanges with the atmosphere may vary according to climate, soil, vegetation, management and global environment (e.g. atmospheric CO<sub>2</sub> and O<sub>3</sub> concentrations). Moreover, horizontal transfers of organic carbon to or from grassland plots may occur as a result of harvesting grass as silage or hay, on the one hand, and of farm manure applications on the other (Soussana et al., 2004). Soil carbon sequestration (enhanced sinks) is the mechanism responsible for most of the mitigation potential in the agriculture sector. Soil C sequestration by the world's permanent pastures could potentially offset up to 4% of the global greenhouse gas emissions (Lal, 2004). Carbon sequestration in grasslands can be determined directly by measuring changes in soil organic carbon (SOC) stocks and indirectly by measuring the net balance of C fluxes. A literature search shows that grassland C sequestration reaches on average  $5\pm30$  g C m<sup>-2</sup> per year according to inventories of SOC stocks and -231 to 77 g C m<sup>-2</sup> per year for drained organic and mineral soils, respectively, according to C flux balance (Soussana et al., 2007; 2010). Off-site C sequestration occurs whenever more manure C is produced by a grassland plot than returned to it. The sum of on- and off-site C sequestration reaches 129, 98 and 71 g C m<sup>-2</sup> per year for grazed, cut and mixed European grasslands on mineral soils, respectively, however with high variability. Direct emissions of  $N_2O$  from soil and of  $CH_4$  from enteric fermentation at grazing, expressed in CO<sub>2</sub> equivalents, offset 10 and 34% of the on-site grassland C sequestration, respectively. Digestion inside the barn of the harvested herbage leads to further emissions of  $CH_4$  and  $N_2O$  by the production systems, which were estimated at 130 g CO<sub>2</sub> equivalents m<sup>-2</sup> per year. The net balance of on- and off-site C sequestration, CH<sub>4</sub> and N<sub>2</sub>O emissions reached 38 g CO<sub>2</sub> equivalents m<sup>-2</sup> per year, indicating a non-significant net sink activity. This net balance was, however, negative for intensively managed cut sites indicating a source to the atmosphere (Soussana et al., 2010).

At the scale of continental Europe, grassland C sequestration has been estimated at  $85\pm12$  M tons C per year and has therefore a strong potential to partly mitigate the GHG balance of ruminant production systems (Schulze *et al.*, 2009). However, as soil C sequestration is both reversible and vulnerable to disturbance, biodiversity loss and climate change, CH<sub>4</sub> and N<sub>2</sub>O emissions from the livestock sector need to be reduced and current SOC stocks preserved. A range of management practices reduce C losses and increase C sequestration: (i) avoiding soil tillage and the conversion of grasslands to arable use, (ii) using light

grazing instead of heavy grazing, (iii) increasing the duration of grass leys; (iv) moderately intensifying nutrient-poor permanent grasslands (Soussana *et al.*, 2004).

Converting a semi-natural grassland used for forage production and grazing to bioenergy production will change the soil carbon balance both through changes in net primary productivity and by altering photosynthetic carbon returns to the soil through plant litter and through rhizodeposition. Some studies have shown carbon sequestration in soils grown for bioenergy Miscanthus crops (Yazaki et al., 2004; Toma et al., 2011). Moreover, Tilman et al. (2006) have shown a large potential for soil carbon sequestration with extensive and species diverse semi-natural grasslands that could potentially be used for bioenergy production. A similar result, regarding soil carbon sequestration, has recently been obtained in Europe with the Jena Biodiversity experiment (Steinbeiss et al., 2008). Therefore, although our knowledge from the GHG balance of European grasslands used for bioenergy production is scarce, the first reports tend to show that carbon sequestration together with fossil fuel energy substitution could add to the greenhouse gas abatement potential of these systems. Nevertheless, these first results need to be confirmed by experiments studying the carbon and GHG budgets of grasslands shifted from livestock to bioenergy production, since replacing grazing by cutting and planting bioenergy grasses such as *Miscanthus* may have impacts on the soil carbon balance that could negate carbon sequestration.

### Overview on major technical concepts of energy production from grassland

This chapter gives an outline on the two most common conversion processes, i.e. biogas production from grass silage and combustion of hay, as well as on the "Integrated Generation of Solid Fuel and Biogas from Biomass (IFBB)" which is a conversion concept just being launched commercially and was especially developed for semi-natural grasslands with one or two cuts per year. Information will be given on techniques available, yields, conversion efficiency, as well as on risks and constraints of the technical concepts.

#### Anaerobic digestion

Surveys of agricultural biogas plants in Germany and Austria show, that grass silage is used as a feedstock in at least 50% of the biogas plants and is the second most frequent crop feedstock after maize silage (Weiland, 2006).

Although crop yield of grassland can be as high as that of a maize crop, in most cases it is significantly lower. The feedstock-specific methane yield of intensively managed grassland biomass is on a similar level (290–320  $l_N$  kg<sup>-1</sup> organic dry matter (oDM)) to maize silage (290–340  $l_N$  kg<sup>-1</sup>oDM) (Weiland, 2010), but there are many management factors affecting feedstock quality, with vegetation, management intensity, particle size and ensiling as the most important (Prochnow, 2009a). Since grassland usually exists as a mixture of various species of grasses, legumes and herbs, it is difficult to draw conclusions on the species effects on feedstock-specific methane potential. There only exists scattered information on pure stands of grass species which consistently show that the influence of grass species on feedstock-specific methane yields can be considered as subordinate to other factors (Baserga and Eggert, 1997; Mähnert, 2002). Advanced maturity, with increased contents of lignin, results in lower feedstock-specific methane yields (Lemmer and Oechsner, 2002; Richter *et al.*, 2011; Figure 1), similar to the reduction in digestibility that occurs when more mature grass is fed to ruminants.

There is some proof that reduced particle size results in higher feedstock-specific methane yields, as the bacteria have a better access to the digestible parts of the substrates (Kaparaju *et al.*, 2002). However, the extent of increase was inconsistent for grasses and other substrates (Prochnow, 2009a). Therefore, for grassland at common management intensity, it remains



Figure 1. (A) Feedstock-specific methane yield and (B) area-related methane yield of grassland silage in whole crop digestion (WCD; 15l batch digester) and of press fluids generated without (PF-hc) and with (PF+hc, mean value with s.e. of mean) hydrothermal conditioning at eight consecutive sampling dates in a spring growth of an unfertilized sub-montane hay meadow (Arrhenaterion) on a loamy soil in Northern Hessen, Germany (from Richter *et al.*, 2011)

uncertain if the additional demand in energy for comminution can be justified through an adequate increase in feedstock-specific methane yields. As the most common digester technique for biogas production is a continuously-stirred reactor (Weiland, 2010), grassland usually is conserved by ensiling which allows a continuous feeding of the reactor along with relatively low substrate losses and appropriate nutrient and water contents (Lehtomäki, 2006). Beneficial effects of additives (microbes, acids, enzymes, sugar or mixtures of them) as reported by the latter author, were not confirmed by other studies (Pakarinen *et al.*, 2008) and costs of application should be considered against the increase in methane yield.

Since Life Cycle Assessment (LCA) studies of biogas production and utilization differ in terms of the scope and the method for impact assessment, comparisons between biogas plants and reference systems need to be drawn carefully. Based on data from 10 full-scale biogas plants located on farms in different regions of Bavaria (Germany), Bachmeier et al. (2010) analysed GHG emissions and resource demand for electricity production from biogas. In comparison to the fossil fuel reference system (electricity production by 30% natural-gas-fired power plants and 70% coal-fired power plants), electricity production in the biogas plants saved GHG emissions by from 573 to 910 g CO<sub>2-eq</sub> kWh<sub>el</sub><sup>-1</sup>. He concluded that in the future, the validity of GHG balances should be enhanced by the provision of reliable data on nitrous oxide emissions from energy crop cultivation, methane leakage from biogas plants and emissions from uncovered storage tanks in comparison to conventional manure management. Although GHG balances show clearly positive results, at least for optimised biogas plants, in many regions with a high density of biogas plants, socio-economic problems due to increased prices of feedstock and land rents occur. Ethical considerations and ecological effects will increase, if land use changes to produce food in vulnerable ecosystems of the tropics are induced by the increased energy crop production in developed countries.

#### Hay combustion

If combustion is chosen for converting biomass into energy, fuel quality becomes of great importance. It is a major determinant for the life span of the combustion plant and for the choice of type of plant (regarding combustion chamber layout, equipment with filter and condensation units, grate methods, etc.). While the carbon (C), hydrogen (H) and

oxygen (O) content in wood from spruce accounts for about 99% of the total biomass, it makes up 95% of the biomass from *Miscanthus* and only 90–93% of grassland biomass depending on the species or species composition (Hartmann, 2009). The remaining fraction consists of elements that contribute to ash formation and/or become volatile and cause emissions. The major ash forming elements in biomass are silicon (Si), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na) and phosphorus (P). These ash forming elements, when exposed to high temperature, either contribute to the melting and deposition of ash on the surface of the combustion chamber (slagging) or contribute to vaporization and subsequently condense in the cooler regions (fouling). However, some elements play multiple roles in these processes which cannot be easily disentangled. The ash softening temperature (AST), the temperature at which a cube of pressed ash shows the first signs of softening, is, according to Hartmann (2009), relatively high for wood (1426°C for spruce) and significantly lower for hay from landscape management (1061°C) (Table 1).

Table 1. Mean, minimum (min) and maximum (max) values of energy content and combustion relevant parameters from different grassland experiments compared with values from *Miscanthus* and spruce wood as two types of biomass frequently used for combustion

· ·									
	HHV	Ν	Ash	Κ	Ca	Mg	Cl	S	
	MJ kg <sup>-1</sup>			a l	ro-LD	м			°C
	DM			gı	kg · D	IVI			C
mean	18.1	18.7	93	17.7	15.2	2.7	3.0	2.3	1248
min	16.3	8.9	53	7.6	2.9	0.8	0.9	1.1	1031
max	19.2	38.4	220	34.1	37.1	7.2	9.2	4.9	1679
mean	-	13.4	84	15.0	10.8	2.3	4.1	2.2	_
min	18.2	10.3	61	8.4	6.7	1.1	1.7	1.5	_
	10.1	174	00	24.2	14.0	27	11.2	25	
шах	19.1	17.4	99	24.2	14.9	5.7	11.2	5.5	_
mean	18.5	12.0	79	-	_	_	2.7	1.6	_
min	17.7	7.0	59	_	_	_	0.8	0.7	_
max	19.5	22.0	118	_	_	_	7.6	3.4	_
mean	17.8	13.7	_	12.0	10.0	3.2	4.8	1.6	1111
min	-	_	_	_	_	_	_	_	_
max	_	_	_	-		_	_	_	_
mean	19.1	7.3	39	7.2	1.6	0.6	2.2	1.5	973
) mean	20.2	1.3	6	1.3	7.0	0.8	0.1	0.2	1426
	mean min max mean min max mean min max mean en mean	HHV MJ kg <sup>-1</sup> DM   mean 18.1   min 16.3   max 19.2   mean -   min 18.2   max 19.1   mean 18.5   min 17.7   max 19.5   mean 17.8   min -   max -   mean 19.1   wean 19.1   wean 19.1	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

HHV - Higher heating value; AST - Ash softening temperature.

Amongst others, the elements creating harmful emission and corrosion problems are nitrogen (N), chlorine (Cl) and sulphur (S). N concentration in the solid fuel shows a logarithmic correlation with the NO<sub>x</sub> emissions (van Loo and Koppejan, 2008), which have been identified as one of the major environmental impacts of solid biomass combustion (Nussbaumer, 2003). Cl is responsible for the formation of HCl and together with alkali metals for the formation of chlorides such as KCl or NaCl. Furthermore, high Cl concentrations are expected to contribute to the formation of polychlorinated dibenzodioxines and dibenzofuranes (PCDD/F) and their concentrations were found to be higher during the combustion of herbaceous biomass than during wood combustion (Launhardt and Thoma, 2000). S contained in the biomass will mainly transform into SO<sub>x</sub>. While 40–90% of the S concentration is bound in the ash, the rest is emitted as SO<sub>2</sub> and to a minor extend as SO<sub>3</sub> (van Loo and Koppejan, 2008). As part of the SO<sub>x</sub> emission process some of the SO<sub>2</sub>/SO<sub>3</sub> will react with deposit alkali chlorides and replace and consequently release Cl as gaseous Cl<sub>2</sub>. This leads to the formation of alkali sulphates while the Cl can repeatedly react with metal parts of the plant forming e.g.  $\text{FeCl}_2$  which is the source of severe corrosion (Riedl *et al.*, 1999). In grasslands, where a multitude of species occurs, fuel quality can vary considerably depending on the individual species composition.

While perennial energy grasses such as switchgrass, *Miscanthus* or reed canary grass have been the focus of research on solid biofuels from grassland biomass (Christian *et al.*, 2002), there is a lack of knowledge on semi-natural grasslands and their characteristics regarding biofuel production (Prochnow *et al.*, 2009b). So far, energy content and fuel quality of grassland biomass have been studied over a range of different locations (Florine *et al.*, 2006; Wachendorf *et al.*, 2009; Tonn *et al.*, 2010) creating insight into the effects of species composition and abiotic location properties on energy content and fuel quality. However, none of the above-mentioned studies had clear diversity gradients that could have attributed their results specifically as diversity effects. Florine *et al.* (2006) mentioned that the variance in chemical composition was due to the diversity of herbaceous plant species, while Tonn *et al.* (2010) found the difference between grasses and herbaceous species to be mainly responsible for the variance. To summarize, cuts should be taken as late as possible in order to minimize nutrient contents in the fuel and fertilization should be kept at a low level. However, furnace technique needs to be adapted to prevent damage and to reduce emissions to an acceptable level.

#### **Integrated Generation of Solid Fuel and Biogas from Biomass**

With the aim of overcoming the problems inherent to anaerobic digestion and direct combustion of grassland biomass, the Integrated Generation of Solid Fuel and Biogas from Biomass (IFBB) system was developed (Wachendorf et al., 2009; Bühle et al., 2011), which separates the easily digestible constituents of grass silage into a liquid form, to be converted into biogas, whilst the more fibrous parts are processed to form a solid fuel (Figure 2). The first step is a hydrothermal conditioning of the silage in which silage and water are mixed and heated under continuous stirring for a short time. This treatment is designed to macerate cell walls and produces a mash which is then mechanically dehydrated by a screw press. As an effect of the conditioning and dehydration, several minerals (e.g. K, Mg, P and Cl) and organic compounds (e.g. carbohydrates, proteins and lipids) are partly transferred into the press fluid (PF), which is an excellent feedstock for biogas production, with specific methane yields of up to 500 normal litres per kg volatile solids (Bühle et al., 2012a; Richter et al., 2009). The remaining press cake (PC) is rich in cellulose and hemicellulose, and contains relatively low concentrations of detrimental elements, which results in an improved quality as a biofuel (Richter et al., 2010). The PC achieves DM concentrations of about 450-500 g kg<sup>-1</sup> through mechanical dehydration, but needs to be dried to a DM content of about 850 g kg<sup>-1</sup> in order to be suited for pelleting and storage. Thus, the IFBB process has a year-round demand for heat produced in the CHP from PF biogas.

In a recent study, with a botanically well-defined sward sampled on consecutive dates over a prolonged spring growth and with hydrothermal conditioning temperatures of 10 to 90°C, mass flows of N, as a major source for NO<sub>x</sub> emissions during combustion, were 0.42 to 0.66. N mass flow, which describes the proportion of total N that goes into PF, is higher in younger plant tissues than in older plant tissue (Wachendorf *et al.*, 2009). This is due to the much higher concentrations of non-protein N and soluble N in young plant tissue than in mature tissue, where N occurs predominantly in structurally insoluble proteins. In contrast to N, K and Cl is predominantly found in the cell as ions in the vacuole and in the cytosol of plant cells. Therefore, both elements are highly mobile, even in mature plant tissue, and more than 80% of these elements can easily be transferred into the PF



Figure 2. Flow chart of the integrated generation of solid fuel and biogas from biomass (IFBB). CHP refers to a combined heat and power plant

during mechanical dehydration. Compared with woodchips of spruce, where Cl, N and S concentrations are well below the critical threshold, concentrations in PC were below the threshold regarding Cl and S, but above it regarding N. In general, conditioning significantly enhanced the reduction in ash and single mineral elements compared to dehydration without prior conditioning. Richter *et al.* (2010) found that the highest fuel quality, i.e. defined as the press cake with the lowest concentrations of elements detrimental for combustion in IFBB-PC, was obtained with a combination of high temperatures of hydrothermal conditioning, low levels of DM and high levels of NDF in the silage.

Ash softening temperatures, as estimated according to Hartmann (2001), are close to the range for woodchips from willow (1200–1300°C). In any case, combustion of IFBB fuels needs an adapted furnace design with a staged combustion process and air supply, which may not be appropriate for small-scaled heating of single private households. Regarding their energy properties, IFBB PC from grassland was found to be between straw (18.5) and wood from short rotation coppice (18.5 to 19.8 MJ kg<sup>-1</sup> DM (Hartmann, 2001). Richter *et al.* (2010) calculated that around 0.50 of the gross energy contained in the biomass from semi-natural grassland was converted into electricity (6%) and heat (94%) through the IFBB process, whereas only 0.25 was recovered through conventional whole-crop fermentation, even when all waste heat from the CHP was utilized.

Multiple regression analysis showed that the main parameters influencing energy output from PF and PC and energy conversion efficiency of the IFBB system were the temperature of hydrothermal conditioning as well as the NDF and DM concentrations of the silage. These factors also influenced mass flows of various plant compounds into the PF and concentrations of elements in the press cake (Richter *et al.*, 2011). The regression model suggests a maximum total energy conversion efficiency at a conditioning temperature of 50°C. At lower temperatures more DM is directed into the PC, increasing the heat demand for drying it, which eventually leads to a reduction in total energy conversion efficiency, as the internal heat supply from biogas combustion for drying becomes deficient at the same time. Concerning DM in the silage, a compromise between solid fuel quality and total conversion efficiency may be possible at high NDF concentrations in the silage (late harvest dates), where the effect of DM was less pronounced and even treatments with low DM concentration in the silage achieved high conversion efficiencies.

There is increasing evidence that the IFBB technique does not provide much advantage over the conventional biogas technique when applied to physiologically young biomasses (Richter *et al.*, 2010; Graß *et al.*, 2009). This applies particularly if all the heat produced with the conventional biogas technique is utilized. However, as the technique proved feasible for the conversion of municipal green cut (Hensgen *et al.*, 2011), it may be a useful add-on facility to existing sewage and green waste treatment plants increasing the range of bio-resources for a renewable supply of energy for municipalities

#### Greenhouse gas balances of energetic use of biomass from semi-natural grasslands

Based on data from 10 European grasslands, life cycle assessment (LCA) analysis was conducted to evaluate the energy and environmental performance of 7 relevant management and utilization systems of semi-natural grasslands: 1) energy recovery by the IFBB technology as a stand-alone system (IFBB-SA), 2) energy recovery by the IFBB technology as an add-on system to an agricultural biogas plant (IFBB-AO), 3) energy recovery by dry fermentation (DF; uses unstirred reactors for biomasses with moisture levels between 75–80%), 4) energy recovery by hay combustion (HC), 5) animal-based utilization by beef cattle husbandry (BC), 6) mulching of the grassland (MU) and 7) composting (CO) (Figure 3). The underlying data are displayed in detail by Bühle *et al.* (2012b).

Gross savings of greenhouse gases amount to 4.6 t CO2-eq ha-1 for the IFBB scenarios and, as mentioned for primary energy savings, are mainly formed by heat supply. Within IFBB-SA, 54.0% of total greenhouse gas emissions result from heat supply for drying of the press cake. Net greenhouse gas savings add up to 2.9 t CO2-ea ha-1. Performance of net greenhouse gas savings of the IFBB system can be improved by the add-on scenario (3.6 t CO<sub>2-eq</sub> ha<sup>-1</sup>). Gross greenhouse gas savings of the dry fermentation system are overall lower and consist of savings of fossil power production for the most part and furthermore of fossil heat and fertiliser savings, as a result of higher recycling rates of organic residues compared to IFBB. Greenhouse gas emissions are similar to the IFBB add-on scenario and the net greenhouse gas savings account for 1.2 t CO<sub>2-eq</sub> ha<sup>-1</sup>. Net savings potentially can be increased to 1.7 t CO<sub>2 en</sub> ha<sup>-1</sup> in case of full heat exploitation of the CHP. HC shows lowest emissions of greenhouse gases among the energy recovery systems. On the other hand, exclusively thermal use of the biomass leads to only marginal credits for fertiliser substitution. HC's net greenhouse gas savings (2.9 t CO<sub>2-eq</sub> ha<sup>-1</sup>) are in the same range as the IFBB stand-alone scenario. The use of extensive grassland by beef cattle incurs high emissions resulting from ruminant digestion, which add up to 75.6% of the total greenhouse gas emissions. Credits of replaced beef cattle husbandry which is based on intensively managed grasslands are similar to the emissions of the extensive system, meaning that the environmental impact in terms of greenhouse gases is almost equivalent for the two procedures. Even though the product (i.e. supply of metabolisable energy) specific methane emissions are lower in the intensive system, the nutrient transfer from the extensive grassland to another land use system and its resulting greenhouse gas credit leads to an almost equalised balance. The overall net greenhouse gas saving is marginal and amounts to 0.1 t CO<sub>2-eq</sub> ha<sup>-1</sup>. If the fertiliser credit for the extensive system would be disregarded, there would be a net greenhouse gas contribution of 0.3 t CO<sub>2-eq</sub> ha<sup>-1</sup>. The mulching system shows considerable emissions of nitrous oxide which are released during the decomposition of the litter. The composting system obtains credits for fertiliser substitution, but is also connected with emissions during the rotting process such as methane and nitrous oxide, which eventually exceed the gross savings, resulting in a net contribution to global warming of 1.2 t CO<sub>2-eq</sub> ha<sup>-1</sup>. It has to be kept in mind, that in general, but particularly for the MU and CO system, the assumptions made for  $N_2O$  and  $CH_4$  emissions have a strong impact on the results, however these data are connected with high uncertainties.



Figure 3. Greenhouse gas balance of the energy recovery by the IFBB as a stand-alone and as an add-on system, as compared to an agricultural biogas plant, dry fermentation, hay combustion, animal-based utilisation by beef cattle husbandry and non-refining systems mulching and composting (from Bühle *et al.*, 2012b)

# Conclusions

Grassland ecosystems may act as a source or a sink of GHG in  $CO_2$  equivalents, depending on their management as well as on soil, climate and atmospheric  $CO_2$  factors. With the current combination of these factors, European grasslands are on average sequestering carbon, thereby contributing to the mitigation of GHG emissions by ruminant farming systems. The conversion of parts of these grasslands to bioenergy production systems would have impacts on their GHG balance which remain to be further investigated by experiments studying the carbon and GHG budgets of grasslands shifted from livestock to bioenergy production. While improved biogas production from residual biomasses allows for net GHG savings, this technique is frequently used to convert intensively managed grasslands, displacing food production elsewhere, which may eventually lead to land use changes in vulnerable, previously extensively or not used landscapes, like e.g. rainforests in the tropics and sub-tropics. Combustion allows higher conversion efficiencies especially with semi-natural grasslands which remain increasingly unused. Innovative conversion techniques, like IFBB which achieve high efficiencies through separation of liquid and solid parts, provide fuels with improved quality and reduced risks of corrosion and emission. Thus, through an energetic use of such grassland no displacement of food production is implemented leading to less socio-economic effects and superior GHG savings. However, fuel quality is sub-optimal for common furnaces and needs technical adaptations or pre-treatment of the parent material.

#### References

Anonymous (2009) Cross compliance – Council Regulation (EC) No 73/2009. Verified February 2012. http://marswiki.jrc.ec.europa.eu/wikicap/index.php/Cross\_Compliance.

Bachmaier J., Effenberger M. and Gronauer A. (2010) Greenhouse gas balance and resource demand of biogas plants in agriculture. *Engineering in Life Science* 10, 560–569.

Baserga U. and Eggert K. (1997) Vergärung von Energiegras zur Biogasgewinnung. Bundesamt für Energiewirtschaft, Forschungsprogramm Biomasse, Tänikon.

Bühle L., Stülpnagel R. and Wachendorf M. (2011) Comparative life cycle assessment of the integrated generation of solid fuel and biogas from biomass (IFBB) and whole crop digestion (WCD) in Germany. *Biomass and Bioenergy* 35, 363–373.

Bühle L., Reulein J., Stülpnagel R., Zerr W. and Wachendorf M. (2012a) Methane yields and digestion dynamics of press fluids from mechanically dehydrated maize silages using different types of digesters. *Bioenergy Research* (DOI 10.1007/s12155-011-9127-5).

Bühle L., Hensgen F., Donnison I., Heinsoo K. and Wachendorf M. (2012b) Life cycle assessment of the integrated generation of solid fuel and biogas from biomass (IFBB) in comparison to different energy recovery, animal-based and non-refining management systems. *Bioresource Technology* (accepted for publication) (DOI 10.1016/j.biortech.2012.02.072).

Clergue B., Amiaud B., Pervanchon F., Lasserre-Joulin F. and Plantureux S. (2005) Biodiversity function and assessment in agricultural areas. A review. *Agronomy for Sustainable Development* 25, 1–15.

Christian D.G., Riche A.B. and Yates N.E. (2002) The yield and composition of switchgrass and coastal panic grass grown as a biofuel in Southern England. *Bioresource Technology* 83, 115–124.

Florine S.E., Moore K.J., Fales S.L., White T.A. and Lee Burras C. (2006) Yield and composition of herbaceous biomass harvested from naturalized grassland in southern Iowa. *Biomass and Bioenergy* 30, 522–528. Freibauer A., Rounsevell M.D.A., Smith P. and Verhagen J. (2004) Carbon sequestration in the agricultural soils of Europe. *Geoderma* 122, 1–23.

Graß R., Reulein J., Scheffer K., Stülpnagel R. and Wachendorf M. (2009) Die integrierte Biogas- und Festbrennstofferzeugung aus Ganzpflanzensilagen. *Berichte über Landwirtschaft* 87, 43–64.

Hartmann H. (2009) Brennstoffzusammensetzung und -eigenschaften. In: Kaltschmitt M., Hartmann H. and Hofbauer H. (eds). *Energie aus Biomasse: Grundlagen, Techniken und Verfahren*, Springer, Berlin, Germany, pp. 333–374.

Hensgen F., Richter F. and Wachendorf M. (2011) Integrated generation of solid fuel and biogas from green cut material from landsape conservation and private households. *Bioresource Technology* 102, 10441–10450. Isselstein J., Jeangros B. and Pavlu V. (2005) Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe. A review. *Agronomy Research* 3, 139–151.

Kaparaju P., Luostarinen S., Kalmari E., Kalmari J. and Rintala J. (2002) Co-digestion of energy crops and industrial confectionery by-products with cow manure: batch-scale and farm-scale evaluation. *Water Science and Technology* 45, 275–280.

Lal R. (2004) Soil carbon sequestration impacts on global climate change and food security. *Science* 304, 1623–1627.

Launhardt T. and Thoma H. (2000) Investigation on organic pollutants from a domestic heating system using various solid biofuels. *Chemosphere* 40, 1149–1157.

Lehtomäki A. (2006) *Biogas production from energy crops and crop residues*. Dissertation, Jyväskylä Studies in Biological and Environmental Science 163, 91 pp., (verified December 2011). <a href="http://dissertations.jyu.fi/studbiol/9513925595.pdf">http://dissertations.jyu.fi/studbiol/9513925595.pdf</a>>.

Lemmer A. and Oechsner H. (2002) Use of grass or field crops for biogas production. In: Proceedings of the AgEng 2002, Paper No. 02-SE-007 (CD-Version), Budapest.

Loo van S. and Koppejan J. (2008) *The handbook of biomass combustion and co-firing*, Earthscan, London, Sterling, VA, USA, 442 pp.

Mähnert P. (2002) *Futtergräser als Kosubstrat für die Biomethanisierung*, Diploma Thesis, Humboldt-Universität zu Berlin, Germany.

Marriott C., Fothergill M., Jeangros B., Scotton M. and Louault F. (2004) Long-term impacts of extensification of grassland management on biodiversity and productivity in upland area. A review. *Agronomie* 24, 447–61.

Nussbaumer T. (2003) Combustion and Co-combustion of Biomass: Fundamentals, technologies, and primary measures for emission reduction. *Energy & Fuels* 17, 1510–1521.

Pakarinen O., Lehtomaki A., Rissanen S. and Rintala J. (2008) Storing energy crops for methane production: effects of solids content and biological additive. *Bioresource Technology* 99, 7074–7082.

Prochnow A., Heiermann M., Plöchl M., Linke B., Idler C., Amon T. and Hobbs P.J. (2009a) Bioenergy from permanent grassland. A review: 1. Biogas. *Bioresource Technology* 100, 4931–4944.

Prochnow A., Heiermann M., Plöchl M., Amon T. and Hobbs P.J. (2009b) Bioenergy from permanent grassland. A review: 2. Combustion. *Bioresource Technology* 100, 4945–4954.

Richter F., Graß R., Fricke T., Zerr W. and Wachendorf M. (2009) Utilization of semi-natural grassland through integrated generation of solid fuel and biogas from biomass. II. Effects of hydrothermal conditioning and mechanical dehydration on anaerobic digestion of press fluids. *Grass and Forage Science* 64, 354–363. Richter F., Fricke T. and Wachendorf M. (2010) Utilization of semi-natural grassland through integrated generation of solid fuel and biogas from biomass. III. Effects of hydrothermal conditioning and mechanical dehydration on solid fuel and biogas from biomass. III. Effects of hydrothermal conditioning and mechanical dehydration on solid fuel properties and on energy and greenhouse gas balances. *Grass and Forage Science* 65, 185–199.

Richter F., Fricke T. and Wachendorf M. (2011) Influence of sward maturity and pre-conditioning temperature on the energy production from grass silage through the integrated generation of solid fuel and biogas from biomass (IFBB): 2. Properties of energy carriers and energy yield. *Bioresource Technology* 102, 4866–4875. Riedl R., Dahl J., Obernberger I. and Narodoslawsky M. (1999) Corrosion in fire tube boilers of biomass combustion plants. In: China Chemical Anticorrosion Technology Association (CCATA) (ed.). *Proceedings of the China Internationnal Corrosion Control Conference '99.* Beijing, China.

Schulze E.D., Ciais P., Luyssaert S., Freibauer A., Janssens I.A., Soussana J.F., Smith P., Grace J., Levin I., Thiruchittampalam B., Heimann M., Dolman A.J., Valentini R., Bousquet P., Peylin P., Peters W., Roedenbeck C., Etiope G., Vuichard N., Wattenbach M., Poussi Z., Nieschulze J., Gash J.H. and the CarboEurope Team (2009) The Greenhouse Gas Balance of Europe: Methane and nitrous oxide compensate the carbon sink of EU-25. *Nature Geosciences* 2, 842–850.

Soussana J.F., Loiseau P., Vuichard N., Ceschia E., Balesdent J. and Chevallier T. (2004) Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use and Management* 20, 219–30.

Soussana J.F., Allard V. and Pilegaard K. (2007) Full accounting of the greenhouse gas  $(CO_2, N_2O, CH_4)$  budget of nine European grassland sites. *Agriculture, Ecosystems and Environment* 121, 121–134.

Soussana J.F., Tallec T. and Blanfort V. (2010) Mitigating the greenhouse gas balance of ruminant production systems through carbon sequestration in grasslands. *Animal* 4, 334–350.

Steinbeiss S., Beßler H., Engels C., Temperton V.M., Buchmann N., Roscher C. and Kreutziger Y. (2008) Plant diversity positively affects short-term soil carbon storage in experimental grasslands. *Global Change Biology* 14, 2937–2949.

Tilman D., Hill J. and Lehman C. (2006) Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science* 314, 1598–600.

Toma Y., Fernandez F.G. and Sato S. (2011) Carbon budget and methane and nitrous oxide emissions over the growing season in a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido. *Japan Global Change Biology Bioenergy* 3, 116–134.

Tonn B., Thumm U. and Claupein W. (2010) Semi-natural grassland biomass for combustion: influence of botanical composition, harvest date and site conditions on fuel composition. *Grass and Forage Science* 65, 383–397.

Wachendorf M., Richter F., Fricke T., Graß R. and Neff R. (2009) Utilisation of semi-natural grassland through an integrated generation of solid fuel and biogas from biomass I: Effects of hydrothermic conditioning and mechanical dehydration on mass flows of organic and mineral plant compounds, and nutrient balances. *Grass and Forage Science* 64(2), 132–143.

Weiland P. (2006) Biomass digestion in agriculture: a successful pathway for the energy production and waste treatment in Germany. *Engineering in Life Sciences* 6, 302–309.

Weiland P. (2010) Biogas production: current state and perspectives. *Applied Microbiology and Biotechnology* 85, 849–860.

Yazaki Y., Mariko S. and Koizumi H. (2004) Carbon dynamics and budget in a *Miscanthus sinensis* grassland in Japan. *Ecological Research* 19(5), 511–520.

# Use of amenity grasses - status quo and innovations

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## Abstract

Grasses have been utilized by humans to enhance their life and environment for more than 10 centuries. Since that time, grass still adds to the beauty of the surrounding landscape, brings relaxation and comfort to the family, and increases the material value of the home. Presently, there are considerable pressures forcing the amenity grass industry, like its agricultural counterpart, to be more sustainable and sensitive to environmental and human health needs. What we can expect in future will be for example the reduced of completely banned pesticide and insecticide use on golf courses and other public green areas. Due to projected climate change, low input management (less fertilization and cutting etc.) with reduced carbon dioxide emission will be promoted. Use of amenity grasses will also have to face increasing drought events and water shortages. This article presents a short overview of the current use of amenity grasses and considers future trends in research and development.

Keywords: turf, lawn, pitch, Festuca, Lolium, Poa

#### Introduction

True grasses, *Poaceae*, make up one of the largest families of flowering plants, comprising more than 600 genera and approximately 10000 species. These numbers can be misleading, since grasses have by far the widest distribution of all flowering plant families (Darke, 2007). A truly cosmopolitan group, grasses grow on every continent and are part of all the major biomes of the terrestrial world. Grasses are found from mountain tops to seashores, from the Arctic Circle south through temperate and tropical zones to Antarctica (Gibson, 2009). Including the cereals, wheat, corn (maize in Europe), rice, rye, oats, barley, millet, sorghum and sugarcane among its members, the grass family is the most important for human civilization, which owes its spread to the development of grass-based agriculture. Human culture around the world has been shaped by grasses and products made from them (Darke, 2007).

Selected grass species have been used for centuries as so-called amenity grasses to stabilize soils, beautify landscapes, and provide arenas for sports and recreational activities. The majority of grass amenities are from the very beginning closely related to home lawns, which have a huge influence on human health and behavior (Turgeon, 2009). The definition, 'amenity' means benefits of a property whose existence increases the value or desirability of that property. An amenity can be either tangible, such as a swimming pool or gym, or intangible, such as proximity to a local school or supermarket. Therefore, considering the above definition, 'amenity grasses' refers to their particular use, not related to food, forage or renewable energy, but mostly to human well-being. Following Cambridge Learners Dictionary, "...amenity means something that is intended or even necessary to make peoples life more pleasant or comfortable". And it is true that grasses, especially amenity grasses, have been necessary for human well-being for many, many years. Food is essential for survival, however, especially in highly industrialized countries, and most people have lost their appreciation of the social, cultural and economic value. The aim of this presentation is

to give a short overview of the present uses of amenity grasses, mostly in Europe, with future perspectives and consideration of probable interesting inventions and expected restrictions.

#### The functionality of grass species for amenity purposes

Turfgrasses are quite unique among cultivated crops due to so called 'phenotypic plasticity'. Turfgrass genotypes are capable of responding to fluctuating environments and stress by morphological and/or physiological changes. Even narrow leaf, dense and close-cut genotypes may successfully survive due to ability to produce seed in noncompetitive conditions. Cultivated crops generally have reduced phenotypic plasticity compared to their wild progenitors (Morishima and Oka, 1975 after Casler and Duncan, 2003). Due to specific phenotypic plasticity, different grass species can be used for quite diverse applications. Generally, grasses can be divided into three groups of functionality: sports (golf greens and other sport turfs), lawn (landscaping, home lawns) and ecosystem service (nutrient dispersal and cycling, land reclamation, water filtration and purification, carbon sequestration) (Duller et al., 2010). There are ca. 18 amenity grass species of practical use in different applications (Table 1). Species of the highest 'functionality' are Agrostis capillaris, Lolium perenne, Festuca arundinacea and Festuca rubra ssp. rubra. These species are usually predominates and are suitable for many, quite diverse uses. In contrast you can find species of very limited usability such as Puccinellia distans or Poa compressa. It is most likely that the range of species usability increases along with adaptation to a wide range of site conditions. Surprisingly, the 'functionality' of major turf species, as mentioned above does not exactly match overall turf quality and stress performance (Table 2).

	Ν	lajor us	es	М	inor us	es		Niche		Overall
Species	Greens	Sport pitches	Fair- way/ lawns	Tennis/ cricket	Polo/ racing	Low maint.	Land	Shade reclam	Drough	func- nttionality ranking
Agrostis capillaris	**		**	*	*	*	**			9
Lolium perenne		**	**	**	**					8
Festuca arundinacea		**			*	*		*	**	7
Festuca rubra ssp.			*		*	*	*		**	7
rubra			*		Ŧ	Ŧ	4		**	/
Poa pratensis		**	**		*	*				6
Festuca ovina			*			*	**	*	*	6
Deschampsia coespi-		*					**	**		5
tosa		Ŷ					**	ጥጥ		Э
Koeleria cristata						*	*	*	*	4
Festuca rubra ssp.	**		*	*						4
commutata	~~		*	*						4
Festuca rubra ssp.	**		*	*						4
A arostis canina	**									2
Agrostis stolonifera	**									2
Cynosurus cristatus <sup>(1)</sup>		*				*				2
Phleum hertolonii <sup>(1)</sup>			*			*				2
$P_{OA}$ supina <sup>(1)</sup>	*		*							2
Poa trivialis <sup>(1)</sup>								**		2
Poa compressa <sup>(1)</sup>									*	1
Puccinelia distans <sup>(1)</sup>							*			1

Table 1. List of amenity grass species and their uses (after Duller et al., 2010, with modifications)

Explanation: \* - used = 1 point, \*\* - predominant, ranking calculated on following basis = 2 points <sup>(1)</sup> - species added by author.

Turf quality traits	Festuca	Festuca	Festuca	Festuca	Poa	Agrostis	Festuca	Lolium
and stress fasters	OVINA / F.	rubra	rubra	rubra	praten-	stolonif-	arundina-	per-
and stress factors	tracny-	commu-	ssp.tricno-	ssp.	sis	era	сеа	enne
TT 6 114 4 14	рпуна	tata	pnylla	rubra				
Turf quality traits:								
Shoot density	5	5	5	2	3	4	1	2
Fineness of leaf	5	4	4	2	2	3	1	1
Cleanness of cut	5	4	4	4	5	3	1	2
Speed of establish- ment	3	4	3	4	1	2	3	5
Nutrient requirements	4	4	4	3	2	1	2	1
Thatch production	3	3	3	2	2	5	2	1
Mean for quality	4.2	4.0	3.8	2.8	2.5	3.0	1.7	2.0
Reaction to								
stress factors:								
Wear tolerance	2	2	2	2	5	1	4	5
Disease tolerance	3	3	2	4	4	1	4	4
Drought tolerance	4	4	4	3	3	3	5	2
Heat tolerance	3	3	3	3	3	4	5	1
Cold tolerance	2	2	2	2	3	3	2	1
Salt tolerance	5	4	4	3	2	3	4	2
Shade tolerance	4	3	3	3	2	3	3	3
Submersion tolerance	2	2	2	2	2	2	3	1
Mean for stress	3.1	2.9	2.8	2.8	3.0	2.5	3.8	2.4
General mean	3.65	3.44	3.29	2.79	2.75	2.75	2.71	2.19

Table 2. The overall turf quality and stress performance of major turf grass species (after Anonymous, 2011, with modifications)

 $\overline{5 - \text{best or low (for nutrient requirements)}}$ .

Some other amenity grass uses (not listed in Table 1) must also be mentioned here. First are the use of grass species sown in floral mixtures for so called 'amenity landscaping' and 'habitat recreation'. This are, for example: *Alopecurus pratensis*, *Agrostis cestellana*, *Anthoxantum odoratum*, *Briza media*, *Bromus erectus*, *Deschampsia flexuosa*, *Trisetum flavescens* or other minor grass species (Żurek and Sevcikova, 2010).

Other amenity grass uses include ornamental grasses for landscape gardening (flower bed grasses) and floristry (dry flower arrangements). The selection of ornamental grasses for landscaping grows every year. Often it is a matter of fashion for a specific genus (cultivar) or theme color. Grasses can be used as specimen plants in perennial flower beds or are even better in large groupings and mass plantings. Ornamental grasses can be used as 'live screens' (*Arundo donax, Panicum virgatum, Miscanthus* species, etc.) or as colour aspects (blue, red, yellow, etc.). It is obviously not possible to give a list of hundreds of ornamental grass species and varieties in this publication, due to limited space.

# **Beneficial aspects of turf**

The majority of amenity grass applications are devoted to turf use. In addition to providing natural beauty, turf area serves many practical functions. Turf provides a resilient, wear-resistant surface for sports, leisure and a wealth of human activities. Some other turf benefits are listed below (after Beard and Green, 1994; Brede, 2000):

• Soil erosion control and dust stabilization. During a 30-minute, rainstorm (75 1 of water per 1 m<sup>2</sup>), ca. 222 kg of soil per hectare could be eroded from bare soil, while

from ground covered with tall fescue turf the loss was found to be only from 10–62 kg per hectare. Soil and dust stabilization around airport runways and taxiways is important to prolong the operating life of airplane engines (Beard and Green, 1994).

- *Binding and storage of carbon dioxide*. It has been estimated that carbon storage in a well-developed turf area is comparable to that in agroecosystems although less than in forests. The yearly carbon increase in permanent grassland on a sandy loam soil was estimated on 0.52 t C ha<sup>-1</sup> (Reheul *et al.*, 2010).
- *Furnishes oxygen.* Ca. 230 m<sup>2</sup> of turf generates enough oxygen for the ongoing needs of family of four (Brede, 2000).
- *Filters and purifies water*. Surface water runoff losses from tobacco cultivation averaged 6.7 mm ha<sup>-1</sup> per 4 weeks, during growing season, whereas, the surface-water runoff from turf averaged only 0.6 mm ha<sup>-1</sup> per 4 weeks. Losses of N and P from tobacco were 2.34 and 0.48 kg ha<sup>-1</sup> per 4 weeks, respectively while for turf only 0.0012 and 0.002 kg ha<sup>-1</sup> per 4 weeks, respectively (Angle, 1985; Gross *et al.*, 1990 after Beard and Green 1994).
- Organic chemical decomposition. Turf grass area can be designed for the catchment and filtration of runoff waters polluted with Pb, Cd, Cu, Zn, hydrocarbon compounds from oil, grease and fuels, hazardous household and industrial waste etc. This is mostly due to the bacterial population in soil litter, grass clippings and thatch of a turf, which is estimated in the order of 10<sup>9</sup> organisms per cm<sup>-2</sup> of litter surface. These organisms offer one of the most active biological systems for the degradation of trapped organic chemicals and pesticides.
- *Temperature moderation*. In some cases, during hot weather, the difference between grass leaf temperature and that of the surrounding air may reach 3–4°C (Żurek, unpublished data). It has been estimated that the front lawn of a typical residential house provides cooling equivalent to that of a 9 ton air conditioner (a typical home-size central air conditioning unit rates in the 3–4 ton capacity).
- *Noise abatement and glare reduction*. Studies have shown that turfgrass surfaces absorb harsh sounds significantly better than hard surfaces (Brede and Green, 1994).
- Decreased noxious pests, allergy-related pollens and human disease exposure. Closely mown residential lawns reduce the numbers of pests as snakes, rodents, mosquitoes, ticks, chiggers, as well as of plants that often produce allergy-related pollens when they flower. A closely mown lawn around the house also reduces unwanted nuisance insects and disease vectors.
- *A cushion for human safety*. Real grass, unlike advanced, synthetic playing surfaces, has desirable, nonabrasive, cushioning effects that help minimize sports injuries. Green turf ground cover provides a unique, low-cost cushioning effect that reduces injuries to the participant, when compared to poorly or non-turfed soils. Impact absorption values of well covered turf could be two times higher than for bare ground (Policińska-Serwa and Prokopiuk, unpublished data).
- *Increase investment value*. An attractive lawn and landscape not only improves the visual appeal of a house but increases its real estate value from 100 to 200%. It is therefore the 'quantitative' meaning of 'amenity' grass definition.
- *Aesthetic benefits of turf.* Well maintained turf may improve mental health via a positive therapeutic impact, and may also contribute to social harmony and improved productivity (Beard and Green, 1994).
- One of the frequently stated *turf disadvantages* is that it needs lots of *water*. But this is only a public perception, not scientifically accepted truth. High water use from turf areas is mostly related to human decision to irrigate the grass, not to the grass itself. Moreover,

without a drop of water during drought periods, most of the currently used turf grass species survive (Żurek, 2006).

#### Future problems with amenity grasses

Despite current uses we have to face future expectations and regulations, mostly related to environment protection. The following are a few of the newly emerged restrictions and 'problems' which may turn into practice and reality very soon, as well as some resolving suggestions:

1. Reduced or banned pesticide and insecticide use on golf courses. Up till recently, the solution to problems connected with stress resistance of amenity grasses has been mostly through appropriate management practices. Consistently and widely resistant cultivars have not been so far largely developed (Duller *et al.*, 2010). For example, perennial ryegrass resistance for diseases such as red thread (*Laetisaria fuciformis*) have shown no significant improvement in France over 30 years (Table 3).

Table 3. Increase in major turf traits of perennial ryegrass in France in period 1975 – 2005 (after Heijden and Roulund, 2010).

Trait	Improvement in score of traits over 10 years (points)	Significance level
Turf density	0.91	***
Genetic color	0.78	***
Turf quality	0.77	***
Fineness of leaf	0.76	***
Resistance to crown rust	0.55	***
Wear resistance	0.54	***
Resistance to red thread		ns
Resistance to other diseases		ns

This is in accordance with the fact that the disease has only a low heritability and selection is only possible for swards but not for single spaced plants as for much of breeding programmes (Heijden and Roulund, 2010). The presence of endophytic fungi which form a symbiosis with many grass species has recently been associated with both abiotic and biotic stress tolerance (Duller *et al.*, 2010; Wiewióra, 2011). Endophyte fungi grow in the intercellular spaces of the above ground plant tissues, live symbiotically within the grass plant and frequently produces toxins that may confer disease and insect resistance to the host plant. These traits are beneficial for grasses which are grown in areas where insect and disease pressure may cause serious damage to their stands (Wiewióra *et al.*, 2011).

2. Climate change – reduced  $CO_2$  emission during turf management (reduced fertilization, low input management). It is clear that one future focus will be on lower input for amenity grasses used in Europe (Heijden and Roulund, 2010). The requirement for significantly reduced artificial fertilizers as well as reduced irrigation will soon affect many turf users. A promising solution is the use of small leaved white clovers, called Microclover (*Trifolium repens* L. form *microphyllum*) in mixture with grasses. These miniature strains of clover has demonstrated improved winter color and appearance for grass and Microclover mixtures in comparison with standard lawn mixtures (Heijden and Roulund, 2010). Generally, low input (or low maintenance) use of amenity grasses is worthy of interest, not only due to significant cost reduction (Brede, 2000). It is also in accordance with another projected future problem – high greenhouse gas emissions and global warming. Many practices, related to grass management (fertilization, mowing, verti-cutting, irrigation etc.) are also responsible for emissions of  $CO_2$ ,  $NO_x$  and other gasses. Therefore, reducing all management practices, will also reduce costs and adverse environmental effects. There is growing realization that amenity grasses have significant benefits in terms of ecosystem service provision beyond the traditional classification (Duller *et al.*, 2010).

Considering projected global warming, temperature changes will begin to cause genetic shifts in populations, favoring those individuals that respond best to the change (Casler and Duncan, 2003). It was found that along with warming of local climate (+1°C per 30 years), mean heading date of Polish ecotypes was significantly advanced by 11 days for cocksfoot and 10 days per 31 years for smooth-stalked meadow grass (Żurek, 2011). All factors of changing climate will have direct effects on grasses, but the duration of these effects and their impact at the level of the ecosystem is still relatively unknown. In the long term, temperature changes will probably change the geographic adaptation of grass species.

3. Increasing drought events and water shortages. Drought tolerant cultivars of major amenity grasses were always very important for breeders and managers. But it is still not clear that any drought tolerant cultivars exist, even in large turf trails in USA (Thorogood, 2003). On other hand it is guite easy to find significant differences between commercial cultivars, on the single trait basis (Zurek, unpublished data). Perennial ryegrass and red fescue cultivars showed a wide range of variation when tested in pots for drought tolerance. It was concluded that the response of the cultivars to water deficit was significantly related to the intensity of transpiration, indirectly measured by the canopy temperature. Further, it was significantly related to the rate of plant water uptake from soil, also indirectly measured by means of soil water content in pots. What is probably of the major interest, is that drought tolerance is species-dependent. In few cases species with genetically enhanced drought tolerance were used for the improvement of other species. One good example is hybridization between Texas bluegrass (Poa arachnifera Torr.) and Poa pratensis. A few of the resulting cultivars demonstrated enhanced drought tolerance (Read and Anderson, 2003). However, considering world-wide water problems, probably the best way is to look for 'water-saving' cultivars rather than for better drought tolerance. Presently modern cultivars with improved drought resistance retain proper turgor in stress conditions by more intense transpiration. Unfortunately, more intense transpiration during the drought period is compatible with higher transpiration rate under the optimal weather conditions, which results in larger amount of water evapotranspirating from the soil (Rybka and Zurek, 2010). Nothing is currently known about the possibility of producing 'water-saving' amenity grass cultivars. Another option for water problems is to use municipal water, wastewater, storm water or other types of water not suitable for people or animals. The idea is to have more than one water source available for use on a single turf site (Duncan et al., 2009).

4. Improved turf quality in reduced light (sports stadia). Sports stadia are becoming larger and, in consequence, more enclosed and shaded inside. Some species as *Poa supina*, *Festuca arundinacea* or *Deschampsia coespitosa* were shown to have greater shade tolerance than other species tested, but the mechanisms that plants used to cope in shade tend to work against the creation of good turf (Duller *et al.*, 2010). Despite the possibilities of special technical solutions (movable pitch, supplemental lighting, use of growth inhibitors or fans to circulate air over module pitch etc.), nothing better than proper management and frequent re-turfing has been developed so far to solve enclosed stadia problems (Stier and Gardner, 2008).

#### Conclusions

Grasses have different life types, growth forms and other properties. Therefore many grass species have many different uses, whose number may increase in future, according to human needs and expectation. As in the past, also in the future, grasses will play an extremely important role in increasing both environment quality and human well being. It is our responsibility to respond future problems, therefore any innovations and ideas that may mitigate these effects are important.

### References

Anonymous (2011) The professional range of amenity grasses, wild flowers and fertilizers. DLF Trifolium, Seeds & Science. www.dlf.co.uk.

Beard J.B. and Green R.L. (1994) The role of turfgrasses in environmental protection and their benefits to humans. *Journal of Environmental Quality* 23(3), 1–16.

Brede D. (2000) *Turfgrass maintenance reduction handbook: sports, lawns, and golf.* Ann Arbor Press, Chelsea, Michigan, USA, 374 pp.

Casler M.D. and Duncan R.R. (2003) Origins of the Turfgrasses. In: Casler M.D., Duncan R.R. (eds) *Tur-fgrass Biology, Genetics, and Breeding.* John Wiley & Sons., Inc., Hoboken, New Jersey, USA, 5–23.

Darke R. (2007) *The Encyclopedia of Grasses for Livable Landscapes*. Timber Press. Portland, USA, 1–488. Duller S., Thorogood D. and Bonos S.A. (2010) Breeding Objectives in Amenity Grasses. In: Boller B., Posselt U.K. and Veronesi F. (eds) *Fodder Crops and Amenity Grasses. Handbook of Plant Breeding*, vol. 5. Springer Science + Business Media, New York, USA, 137–160.

Duncan R.R., Carrow R.N. and Huck M.T. (2009) *Turfgrass and landscape irrigation water quality. Assessment and management.* CRC Press, Taylor & Francis Group, Boca Raton, London, New York, 464 pp. Gibson D.J. (2009) *Grasses and Grassland Ecology.* Oxford University Press, New York, USA, 1–305.

Heijden A.G. van der and Roulund N. (2010) Genetic gain in agronomic value of forage crops and turf: a review. In: Huyghe Ch. (ed.) *Sustainable use of genetic diversity in forage and turf breeding*. Springer Science + Bussiness Media, New York, USA, 247–260.

Read J.C. and Anderson S.J. (2003) Texas bluegrass. In: *Turfgrass Biology, Genetics, and Breeding*. John Wiley & Sons., Inc., Hoboken, New Jersey, USA, 61–66.

Reheul D., De Cauwer B. and Cougnon M. (2010) The role of forage crops in multifunctional agriculture. In: Boller B., Posselt U.K. and Veronesi F. (eds) *Fodder Crops and Amenity Grasses. Handbook of Plant Breeding*, vol. 5. Springer Science + Business Media, New York, USA, 1–12.

Rybka K. and Żurek G. (2010). Effective use of water as an obligate criterion in plant breeding. *Postępy Nauk Rolniczych (Advances in Agricultural Sciences)* 4, 19–32 (in Polish).

Stier J.C. and Gardner D.S. (2009) Shade stress and management. In: Pessarakli M. (ed.) *Handbook of turfgrass management and physiology*. CRC Press, Taylor & Francis Group, Boca Raton, London, New York, 447–471.

Thorogood D. (2003) Perennial ryegrass. In: Casler M.D. and Duncan R.R. (eds) *Turfgrass Biology, Genetics, and Breeding.* John Wiley & Sons., Inc., Hoboken, New Jersey, USA, 75–105.

Turgeon A.J. (2009) Urban, Suburban, and Rural Amenities of Grass. In: Wedin W.F. and Fales S.L. (eds) *Grassland: Quietness and Strength for a New American Agriculture*. American Soc. of Agronomy, Crop Sci. Soc. of America, Soil Sci. Soc. of America, Madison, USA, 137–154.

Wiewióra B. (2011) Endophytic fungi from Neotyphodium genera occurred in perennial grasses in Poland and their importance for fodder cropping and turf maintenance. *PBAI – NRI Monographs and Dissertations*, 38, 115 pp. (in Polish).

Wiewióra B., Żurek G., Prończuk M., Żurek M. and Schmidt J. (2011) Relations between site conditions and endophyte colonization of grasses in Poland. *Journal of Life Sciences*, 5(10), 831–837.

Żurek G. and Sevcikova M. (2010) Minor Grass Species. In: Boller B., Posselt U.K. and Veronesi F. (eds) *Fodder Crops and Amenity Grasses. Handbook of Plant Breeding*, vol. 5. Springer Science + Business Media, New York, USA, 381–394.

Żurek G. (2006) Grass response to drought stress – evaluation methods and their application to turf species. *PBAI – NRI Monographs and Dissertations* 25, 106 pp. (in Polish).

Żurek G. (2011) Effect of climate change on phenology of forage grass species. *Journal of Life Sciences* 5(9), 754–758.

# Session 3.1. Renewable energy from grass

# Suitability of semi-natural grassland biomass for combustion and the effect of quality optimisation strategies

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# Abstract

Bioenergy generation through combustion offers new opportunities to utilize biomass from species-rich semi-natural grasslands. Chemical composition can limit the suitability of grassland biomass as a fuel through environmentally damaging emissions (high N and ash contents) and problematic ash high-temperature behaviour (high K and Cl, low Ca and Mg contents, causing slagging, fouling and corrosion). Natural variability of biomass fuel quality due to botanical composition and site effects as investigated in a field experiment was compared to the effects of two potential quality optimization strategies: winter harvest and leaching by rain during the field drying period. High natural variability, linked both to botanical composition and site effects, was found for Cl, K, Ca and Mg contents, indicating that it may be possible to select grassland biomass with more favourable ash high-temperature behaviour for combustion. Whilst changes in chemical composition between June and October were small, both winter harvest and leaching strongly decreased K and Cl contents and favourably influenced ash melting behaviour. Ash and N contents showed rather small natural variability and were not strongly influenced by harvest dates or leaching, making it necessary to address emission problems by further improvement of combustion technology.

Keywords: bioenergy, chemical composition, combustion, semi-natural grasslands

# Introduction

Bioenergy generation offers new opportunities to utilize biomass from species-rich semi-natural grasslands. Although combustion is a particularly well-suited conversion technology for mature, fibre-rich grassland herbage, contents of ash and a number of mineral elements can lead to problems during the combustion process. These quality parameters show a high variability due to botanical composition, harvest date and site effects (Tonn *et al.*, 2010). Under certain conditions, strategies that have been used to improve fuel quality of other herbaceous biofuels, such as leaching of unwanted elements by rainfall during the field drying period (Jenkins *et al.*, 1996) or delayed harvest in winter or spring (Landström *et al.*, 2008; 2010). This paper aims to compare the effects of natural variability and of two quality optimization strategies (leaching and delayed harvest) on biomass quality parameters responsible for environmentally damaging emissions during combustion (ash and N content) and for ash-related problems of slagging, fouling and corrosion (K, Cl, Ca and Mg content).

#### Materials and methods

Natural variability and the effects of harvest date were investigated in a field experiment at six semi-natural grassland sites in south-west Germany. At each site, the first growth was harvested monthly from mid-June to mid-October 2007, the period during which the first cut had been made at the various sites previous to the experiment (conventional harvest dates). Harvested biomass
was separated into grasses and forbs (see Tonn *et al.*, 2010). At the same sites, the first growth was harvested at two winter harvest dates, mid-December 2007 and mid-February 2008, without sorting the biomass into grasses and forbs. Influence of leaching was assessed by subjecting oven-dried biomass harvested from random locations at five of the six experimental sites in mid-July and mid-September to two leaching treatments and comparing them to an unleached control. For these treatments, biomass samples were placed on a laboratory shaker in bottles filled with tap water for either 10 or 120 min, corresponding to the effect of 30–40 mm and >70 mm of natural precipitation (Tonn *et al.*, 2011).

All biomass samples were analysed for ash, N, Cl, K, Ca and Mg content (Tonn *et al.*, 2010). Ash melting behaviour and Si contents were investigated for all treatments of the leaching experiment and for thirty-five representative treatments of the field experiment, by heating 200-mg ash samples to 1000°C for four hours and visually classifying them into the four categories 'loose', 'slightly sintered', 'strongly sintered' and 'molten'. To characterize the ash composition of these samples the ratios K/ash, Si/ash and (Ca+Mg)/ash were calculated and normalized to add up to 100%. These are denoted by  $K_a$ ,  $Si_a$  and (Ca+Mg)<sub>a</sub> in the following.

Linear contrasts of log-transformed chemical parameters between the following groups were calculated following an analysis of variance: (a) sites with the respective maximum and minimum content of each element (June-Oct); (b) grasses and forbs (June-Oct); (c) harvest dates June, Oct, Dec and Feb (for June-Oct harvests, whole-plot chemical composition was calculated as the weighted average of that of grass and forb fractions), and (d) leaching treatments and control. Due to back-transformation from the log-scale, these contrasts are expressed as proportions of each first contrast variable (Tonn *et al.*, 2010; 2011).

#### **Results and discussion**

All parameters except N showed marked site differences (Table 1). Ash and elemental contents also differed significantly between grasses and forbs. With the exception of Cl, they were higher in forbs than in grasses, particularly large differences occurring for Ca and Mg. Compared to this variation, changes during the conventional harvest period (June-October) were relevant only for K contents with a mean decrease of 28%.

11							
		Ash	N	Cl	K	Ca	Mg
			Natu	ral variability			
a)	site minimum	71.54	13.20	1.85	8.07	7.61	1.32
	site maximum	93.95	14.45	7.41	18.85	12.09	4.53
	$\Delta$ (site max.–	0.00/0.00	0.01/0.15	0 (0/0 00	0 50/0 60	0.22/0.41	0 (0/0 72
	site min )	0.20/0.28	0.01/0.15	0.68/0.80	0.52/0.62	0.32/0.41	0.68/0.73
b)	forbs	92.08	14.86	2.73	14.85	19.64	3.41
- /	grasses	77.17	12.68	3.52	13.31	5.31	1.51
	$\Delta$ (forbs-grasses)	0.18/0.40	0.14/0.18	-0.12/-0.17	0.13/0.53	0.72/0.74	0.53/0.58
	Q/	c) ()	uality optimizati	on strategy 1: Del	aved harvest		
	June	78.54	14.94	3.97	18.56	9.40	2.26
	Oct	86.00	13.15	3.47	13.38	11.54	2.26
	Dec	81.32	11.02	0.79	3.58	10.19	1.44
	Feb	75.81	10.88	0.36	2.14	9.33	1.18
	$\Delta$ (June–Oct)	-0.05/-0.14	0.08/0.16	0.02/0.22	0.23/0.33	-0.16/-0.30	-0.06/0.06
	$\Delta$ (Oct–Dec)	0.02/0.09	0.12/0.20	0.75/0.80	0.71/0.75	0.06/0.17	0.32/0.40
	$\Delta$ (Oct–Feb)	0.08/0.15	0.13/0.21	0.88/0.91	0.83/0.85	0.14/0.24	0.45/0.51
		d	) Quality optimi	zation strategy 2:	Leaching		
	control	82.24	1.32	2.89	14.61	10.05	2.00
	10 min	73.97	1.29	1.59	10.28	9.64	1.88
	120 min 1	65.39	1.30	0.52	5.33	9.28	1.50
	$\Delta$ (control-10 min)	0.08/0.12	0.01/0.04	0.41/0.49	0.27/0.32	0.02/0.06	0.01/0.11
	$\Delta(\text{control}-12 \text{ min})$	0.19/0.22	0.00/0.03	0.81/0.83	0.62/0.65	0.06/0.10	0.21/0.29

Table 1. Ash and elemental contents of grassland biomass (g kg<sup>-1</sup>), back-transformed group means;  $\Delta$ : linear contrasts between group means expressed as proportion of the first contrast variable, lower and upper limit of 95% confidence interval

Winter harvest in December, however, led to considerably stronger decreases of K and similarly large ones of Cl contents, as well as to somewhat lesser reductions of Mg. The

effect on the other parameters was small. Whilst harvesting in February was associated with much larger biomass yield losses (Tonn *et al.*, 2008), the chemical composition differed little from that in December. Leaching also strongly affected K and Cl contents, but reductions comparable to those achieved by winter harvest were only found in the 120-mm leaching treatment. This corresponded to the effect of >70 mm of rain, which is unlikely to be realized on the field.

Ash composition clearly differed between grasses with high Si<sub>n</sub> and low  $(Ca+Mg)_n$  and forbs with low Si<sub>n</sub> and high  $(Ca+Mg)_n$ , but ash high-temperature behaviour was very variable in both groups. Low values of K<sub>n</sub>, either through winter harvest (Figure 1a) or through leaching (Figure 1b), were most closely connected to favourable ash high-temperature behaviour. For samples with K<sub>n</sub>  $\leq$  26%, no molten or strongly sintered ash was found after heating to 1000°C.



Figure 1. State of grassland biomass ash after heating to  $1000^{\circ}$ C in relation to normalized proportions of K/ash, Si/ash and (Ca+Mg)/ash. (a) field experiment, (b) leaching experiment

#### Conclusions

Ash and N contents showed the smallest natural variability and were not strongly influenced by harvest dates or leaching, making it necessary to address emission problems by further improvement of combustion technology. High natural variability of Cl, K, Ca and Mg contents, on the other hand, allows the selection of grassland biomass with more favourable ash high-temperature behaviour. Contents of Cl and K were also most strongly affected by the two investigated quality optimization strategies, winter harvest and leaching, which could be shown to improve ash melting behaviour. While application of these strategies is limited to specific grassland types or weather conditions, they can considerably reduce ash-related problems, for which technical solutions are often difficult to achieve.

#### References

Jenkins B.M., Bakker R.R. and Wei J.B. (1996) On the properties of washed straw. *Biomass and Bioenergy* 10, 177–200.

Landström S., Lomakka L. and Anderson S. (1996) Harvest in spring improves yield and quality of reed canary grass as a bioenergy crop. *Biomass and Bioenergy* 11, 333–341.

Tonn B., Thumm U. and Claupein W. (2008) Späte Schnittzeitpunkte von Extensivgrünland – eine Strategie zur Erzeugung qualitativ hochwertiger Biofestbrennstoffe aus der Landschaftspflege. *Mitteilungen der Arbeitsgemeinschaft Grünland und Futterbau* 9, 293–296.

Tonn B., Thumm U. and Claupein W. (2010) Semi-natural grassland biomass for combustion: influence of botanical composition, harvest date and site conditions on fuel composition. *Grass and Forage Science* 65, 383–397.

Tonn B., Dengler V., Thumm U., Piepho H.-P. and Claupein W. (2011) Influence of leaching on the chemical composition of grassland biomass for combustion. *Grass and Forage Science* 66, 464–473.

# *Phalaris aquatica* L. lignocellulosic biomass as second generation bioethanol feedstock

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## Abstract

Environmental impacts associated with the use of fossil fuels, rising prices, potential limitations in supply and concerns about regional and national security are driving the development and use of different biomass types for bioenergy and biofuels. One of them, lignocellulosic biomass, is found in abundance in perennial grass species. In our study we investigated at two regions of North Greece with different altitudes, the aboveground biomass production and lignocellulosic content of the perennial grass species *Phalaris aquatica* L. for bioethanol production. Subsequently, *P. aquatica* L. biomass underwent acid and enzymatic hydrolysis in order to release sugar monomers from the cell wall polymer matrix. The results indicated that *P. aquatica* L. could serve as a second-generation bioethanol feedstock in lowland areas of North Greece due to its higher biomass yield and lignocellulosic content than in upland areas. The results also showed that acid pretreatment and enzymatic hydrolysis could convert > 80% of structural carbohydrates to fermentable sugar monomers.

Keywords: perennial grasses, Phalaris aquatica, cellulosic biomass, pretreatment, bioethanol

## Introduction

Cellulosic ethanol production from herbaceous biomass energy crops is an alternative to fossil fuel-based liquid fuels. Lignocellulosic perennial crops such as inedible grasses are promising feedstock because of high yields, low costs, good suitability for low quality land and low environmental impacts (Balat *et al.*, 2008). Lignocellulosic biomass contains structural carbohydrates, such as cellulose and hemicellulose, which are not readily available for bioconversion; hence, a pretreatment step is required. Dilute acid pretreatment followed by enzymatic hydrolysis is the typical process used to convert lignocellulosic biomass to sugar monomers (Iranmahboob *et al.*, 2002). The objective of the present study was to compare biomass yield and lignocellulosic concentration of *Phalaris aquatica* L., at two sites of North Greece with different altitudes, for second-generation bioethanol production.

## Materials and methods

Biomass samples of perennial grass (*Phalaris aquatica* L.) rainfed plantations (10 years old) in North Greece, located at sites (Thermi and Chrysopigi) with different altitudes (30 and 650 m) respectively, were collected at the end of the growing season of 2009. Biomass production was measured using 10 ( $1 \times 1$  m<sup>2</sup>) plots. Harvested biomass was dried at 60°C for 48 h, milled to a size of 1 mm and subjected to fibre analyses (NDF, ADF, ADL) using the Van Soest method (Van Soest *et al.*, 1991). Hemicellulose and cellulose concentrations were determined by the differences of (NDF-ADF) and (ADF-ADL) respectively. Lowland biomass samples at 10% (w/v) solid loading was mixed with 1.5% (w/v) H<sub>2</sub>SO<sub>4</sub> acid and

pretreated at 120°C with reaction time of 45 min in 50-ml screw cap bottles in oil bath. Solid residues recovered after pretreatment by filtration were hydrolysed with cellulases mixture (Celluclast 1.5L and Novozyme 188). Enzymatic hydrolyses were performed in 100-ml flasks with 50 ml of 0.05 M sodium citrate buffer (pH = 4.8) at 50°C, 2% (w/v) solid loading, 40 FPU g<sup>-1</sup> solid enzyme loading for 48 h in an incubator at 150 rpm agitation speed. Total monomeric sugars were analysed by HPLC. All experiments were performed in duplicate. One-way ANOVA was used to compare biomass production and chemical composition of *P. aquatica* at two sites. Further differences were evaluated with the LSD post hoc test, at a level of significance of 0.05 (Kinnear and Gray, 2008). Carbohydrate conversion efficiency was calculated from the ratio of total sugars released from biochemical process and the beginning (hemicellulose + cellulose) content (Shung and Cheng, 2005).

#### **Results and discussion**

The change of biomass yield and lignocellulosic concentration of *P. aquatica* at sites with different altitudes and climatic conditions is presented in Table 1. The lowland site (Thermi) had a significantly higher biomass yield compared to the upland one (Chrysopigi). Similar results were found by McLeod *et al.* (2006) and Culvenor *et al.* (2007) studying *P. aquatica* dry matter yield in a Mediterranean environment of Australia, indicating better adaptability of the species at dry and warm regions. The climate of the Themi site could be characterized as dry and warm with 443 mm mean yearly rainfall and mean temperature of 16.8°C, while at the Chrysopigi site mean temperature decreases and rainfall increase respectively, which determine plant growth. According to Koukoura (2003) *P. aquatica* is a species which mainly spreads at low altitude areas of the Mediterranean climatic regions.

<b>C</b> :4	Mean tem-	Mean	Biomass yield	NDF	ADF	ADL	Soluble cell components	Hemicellulose + cellulose		
Siles	(°C)	(mm)	(Mg ha <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)						
Thermi	16.8	443	6.26a	770a	503a	63a	230a	707a		
Chyso-	13.5	557	4.90b	740b	441b	73a	260b	667b		

Table 1. Mean biomass production and cell wall concentration of *Phalaris aquatica* at sites with different altitude and climatic conditions

a, b – Means followed by the same letter do not differ statistically significant (LSD Test, P < 0.05).

Furthermore, the biomass at the Chrysopigi site had significant lower lignocellulose (NDF) and total structural carbohydrates (hemicellulose + cellulose) concentration than at the Thermi site, but higher water-soluble cell components, where non-structural carbohydrates (WSC) are present. This reduction could be attributed to temperature decrease with altitude, since temperate grasses accumulate water soluble carbohydrates (WSC) as an energy reserve in correspondence with low temperatures (Moriyama *et al.*, 2003) and a relationship between WSC and winter stress factors has been reported from other authors (Hofgaard *et al.*, 2003).

Plant lignocellulosic biomass is a matrix of cross-linked polysaccharide networks, mainly cellulose and hemicellulose, which are tightly bound to lignin. The bioconversion process of lignocellulosic biomass to ethanol includes three main steps: a) pretreatment to solubilize hemicellulose into sugars, b) enzymatic hydrolysis to convert cellulose to glucose and c) fermentation of total released sugars to produce ethanol (Nigam and Singh, 2011). In Table 2 is presented the yield of total sugars released under different stages of *P. aquatica* lignocellulosic biomass conversion process of the Thermi site. Dilute acid pretreatment

solubilize 212 g kg<sup>-1</sup> DM of hemicellulose while under enzymatic hydrolysis 424 g kg<sup>-1</sup> DM of glucose was released from cellulose, accounting for 89.9% of total structural carbohydrate (hemicellulose + cellulose) conversion efficiency to sugars monomers, which can be fermented to ethanol. Dien *et al.*, (2006) reported 80–93% conversion efficiency of total structural carbohydrates to sugar monomers of different perennial grasses (*Panicum virgatum* L., *Phalaris arundinacea* L.) under the same biochemical method, while according to Sun and Cheng (2005) 81.9% of bermudagrass (*Cynodon dactylon* L.) structural carbohydrates were converted to fermentable sugars under mild (121°C) dilute acid H<sub>2</sub>SO<sub>4</sub> pretreatment and enzymatic hydrolysis reaction conditions using the same cellulase mixture.

Table 2. Yield of monomeric sugar under different stages of *Phalaris aquatica* bioconversion processes of the Thermi site

	Dilute acid pretreat- ment	Enzymatic hy- drolysis	Total carbohydrate (hemi- cellulose + cellulose) con- version efficiency (%)
Monomeric sugars (g kg <sup>-1</sup> DM)	212	424	89.9

#### Conclusions

*Phalaris aquatica* is a potential second-generation bioethanol feedstock at lowland sites of North Greece due to higher biomass yield and total structural carbohydrate concentration compared to upland sites. Acid pretreatment followed by enzymatic hydrolysis is an effective biochemical method to release fermentable sugar monomers from *P. aquatica* cell wall carbohydrates for bioethanol production.

#### References

Balat M., Balat H. and Oz C. (2008) Progress in bioethanol processing. *Progress in Energy and Combus*tion Science 34, 551–573.

Culvenor R.A., Boschma S.P. and Reed K.F.M. (2007) Persistence of winter-active phalaris breeding populations, cultivars and other temperate grasses in diverse environments of south-eastern Australia. *Australian Journal of Experimental Agriculture* 47, 136–148.

Dien B.S., Jung H-J.G., Vogel K.P., Casler M.D., Lamb J.F.S., Iten L., Mitchell R.B. and Sarath G. (2006) Chemical composition and response to dilute-acid pretreatment enzymatic saccharification of alfalfa, reed canarygrass and switchgrass. *Biomass and Bioenergy* 30, 880–891.

Hofgaard I.S, Vollsnes A.V., Marum P., Larsen A. and Tronsmo A.M. (2003) Variation in resistance to different winter stress factors within a full-sib family of perennial ryegrass. *Euphytica* 134, 61–75.

Iranmahboob J., Nadim F. and Monemi S. (2002) Optimizing acid hydrolysis: a critical step for production of ethanol from mixed wood chips. *Biomass and Bioenergy* 22, 401–404.

Kinnear P.R. and Gray C.D. (2008) SPSS 15 Made Simple. Psychology Press. Hove.

Koukoura Z. (2003) *The most important range plants in Greece*, Publication Department, Aristotle University of Thessaloniki, Thessaloniki, 55 pp.

McLeod M.K., McLeod D.A. and Daniel H. (2006) The effect of degradation of phalaris + white clover pasture on soil water regimes of a Brown Chromosol on the Northern Tablelands of NSW, Australia. *Agricultural Water Management* 82, 318–342.

Moriyama M., Abe J. and Yoshida M. (2003) Etiolated growth in relation to energy reserves and winter survival in three temperate grasses. *Euphytica* 129, 351–360.

Nigam S.H and Singh A. (2011) Production of liquid biofuels from renewable resources. *Progress in Energy* and Combustion Science 37, 52–68.

Sun Y. and Cheng J.J. (2005). Dilute acid pretreatment of rye straw and bermudagrass for ethanol production. *Bioresource Technology* 96, 1599–1606.

Van Soest P.J., Robertson J.B. and Lewis B.A. (1991) Methods for dietary fiber, Neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583–3597.

# Investigating the effects of hydrothermal conditioning, detergent and mechanical pressing on the isolation of the fibrous press-cake fraction from grass silage

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## Abstract

This study investigated the effects of hydrothermal conditioning with and without detergent, and mechanical pressing, on the isolation of the press-cake (PC) from a range of grass silages. In Experiment 1, perennial ryegrass and cocksfoot silages harvested at two growth stages were subjected to hydrothermal conditioning (3 water: 1 silage) at three temperatures (20, 40 and 60°C) with or without detergent (sodium dodecyl sulphate; 30 g L<sup>-1</sup>). In Experiment 2, Italian ryegrass and timothy silages harvested at three growth stages were subjected to three washing steps (30 minutes at 60°C in 3 water + detergent (30 g L<sup>-1</sup>): 1 silage; repeated 1, 2 or 3 times), before being mechanically pressed (1.5, 3.0 or 4.5 MPa). Repeated washing and high pressure mechanical pressing proved the most effective treatments in removing total solubles from a range of grass silages and thus in isolating a fibre-rich PC fraction. Increasing water temperature and the inclusion of a detergent had little effect on the proportion of total solubles removed or on the NDF concentration of the resulting PC.

Keywords: grass silage, fractionation, cell solubles, hydrothermal conditioning, mechanical pressing

## Introduction

An essential component of the 'Green Biorefinery' concept is the initial fractionation of green biomass into a fibre-rich press-cake (PC) and a nutrient-rich press-juice. The PC is rich in hemicellulose, cellulose, lignin and some pectic substances, whilst the press-juice contains proteins, amino acids, organic acids, water soluble carbohydrates and other substances (Kromus *et al.*, 2006). Despite fractionation being the crucial first step in the process, relatively little information on the factors influencing this practice is published. In this laboratory-based study two experiments were undertaken to investigate the effects of hydrothermal conditioning with and without detergent and mechanical pressing on the isolation of a fibre-rich PC from grass silages differing in botanical species and harvest date.

## Materials and methods

In Experiment 1, perennial ryegrass (PRG; *Lolium perenne* cv. Gandalf) and cocksfoot (*Dactylis glomerata* cv. Pizza) were grown in field plots (each 20 m<sup>2</sup>) to which 125 kg fertiliser N was applied per ha, and harvested at two dates [Harvest 1 (26 May) and Harvest 2 (7 July)] in the primary growth, with triplicate replication. At each harvest date, grass was precision-chopped and representative 6 kg samples were ensiled in laboratory silos. After 100 days ensilage, representative 200 g silage samples were subjected to hydrothermal conditioning (3 solvent (600 ml): 1 silage) at three temperatures (20, 40 and 60°C) for 30 minutes. The solvent was distilled water with or without detergent (sodium dodecyl sulphate; SDS, 30 or 0 g/L, respectively). In Experiment 2, triplicate plots of Italian ryegrass (IRG; *Lolium*)

multiflorum cv. Prospect) and timothy (Phleum pratense cv. Erecta) were grown in field plots as in Exp. 1, but harvested and ensiled at three dates [Harvest 1(12 May), Harvest 2 (9 June) and Harvest 3 (7 July)] in the primary growth. Representative 200 g silage samples were subjected to 3 washing steps (30 minutes at  $60^{\circ}$ C in 3 water + detergent (30 g L<sup>-1</sup>): 1 silage; repeated 1, 2 or 3 times), followed by three mechanical pressing treatments (1.5, 3.0 and 4.5 MPa) using an hydraulic press. Silage and processed PC samples were dried at 85°C for 16 h in an oven to estimate dry matter content (DM), and corrected for the loss of volatiles, while neutral detergent fibre (NDF) and ash were chemically analysed from dried (40°C for 48 h) milled (1 mm screen) samples as described by McEniry et al. (2006). Aqueous juice extracts from both the silage and PC fraction were used to determine total solubles (lactic acid, acetic acid, propionic acid, butyric acid, ethanol, water soluble carbohydrates and ammonia) before and after treatment, respectively. Data for both experiments were analysed as a split-split plot design with harvest as the main plot and grass species as the subplot (with triplicate replication), and with a 3 (temperature)  $\times$  2 (detergent) and a 3 (washing steps)  $\times$  3 (hydraulic pressing) factorial arrangement of treatments at the sub-sub-plot level for Exp. 1 and 2, respectively.

#### Results

In Experiment 1, hydrothermal conditioning with and without detergent resulted in the isolation of a fibre-rich PC (769 g NDF kg<sup>-1</sup> DM in PC compared with 638 g NDF kg<sup>-1</sup> DM in the original silage). Grass species had no effect (P > 0.05) on the DM and NDF concentration of the PC after treatment or on the proportion of solubles removed from silages (data not shown). However, there was a lower proportion of solubles removed (P < 0.01) from the late compared with the early harvest silages (Table 1). In Experiment 2, PC prepared from the timothy silage had a higher DM (P < 0.01) and NDF (P < 0.001) concentration than PC from IRG; however, there was no difference (P > 0.05) in the proportion of solubles

DM)

Table 1. Experiment 1: Harvest date effects on removal of solubles from silage and on press-cake (PC) dry matter (DM) and neutral detergent fibre (NDF) concentrations

<sup>1</sup>Solubles in PC relative to silage prior to processing; <sup>2</sup>Standard error of the mean.

Table 2. Experiment 2: Mech	anical pressure and wasl	hing effects on remov	al of solubles from	n silage
and on press-cake (PC) dry n	natter (DM) and neutral	detergent fibre (NDF	) concentrations	

·		•	•	
Pressure	Washing	Proportion of solubles removed <sup>1</sup>	PC DM (g kg <sup>-1</sup> )	PC NDF (g kg <sup>-1</sup> DM)
1.5	1	0.88	296	742
1.5	2	0.95	278	768
1.5	3	0.94	279	777
3	1	0.90	311	752
3	2	0.95	310	770
3	3	0.96	302	783
4.5	1	0.90	333	757
4.5	2	0.95	328	775
4.5	3	0.96	314	787
SEM <sup>2</sup>		0.007	4.3	3.0
<i>P</i> -value		0.390	0.170	0.418

<sup>1</sup>Solubles in PC relative to silage prior to processing; <sup>2</sup>Standard error of the mean (for interaction).

removed. In general, PC from the later harvested silage had a higher DM (P < 0.001) and NDF (P < 0.01) concentration, while in contrast a lower proportion of solubles were removed (P < 0.05). The proportion of solubles and the PC NDF concentration increased (P < 0.001), while PC DM concentration decreased (P < 0.001), with increasing number of washings (Table 2). The proportion of solubles removed was further enhanced by increasing mechanical pressing (Table 2). Press-cake DM and NDF concentrations also increased (P < 0.001) with increasing mechanical pressing.

#### Discussion

Wachendorf *et al.* (2009) reported higher mass flow rates of plant compounds (e.g. crude protein and minerals) into the press-juice at temperatures of 60 and 80°C (due to a modification of the cell structure) compared to those at a low temperature. This is in contrast to the current study where the three temperatures selected were equally effective in the removal of total solubles and the isolation of a fibre-rich PC fraction. The detergent (so-dium dodecyl sulphate) used in this study was selected based on its function in removing solubles to isolate fibre in the NDF assay. However, the addition of the detergent had no further impact on the removal of total solubles from grass silage and this may have been the result of lower water temperatures compared to that of the NDF assay (100°C). Increasing the number of washings steps enhanced the removal of solubles resulting in the isolation of a more fibre-rich PC fraction. On average, the largest increase in PC NDF was observed between washing step 1 and 2. The mechanical pressure used in the current experiment would appear to be lower than that achieved in industrial screw presses where the DM concentration of the resulting PC would be closer to 400 g kg<sup>-1</sup> (versus 247 to 395 g DM kg<sup>-1</sup> in the current experiment).

#### Conclusions

Repeated washing and high pressure mechanical pressing proved the most effective treatments in removing total solubles from a range of grass silages and thus isolating a fibre-rich PC fraction. Later harvested herbage was more difficult to fractionate, whilst the grass species appeared to have little effect on the fractionation process.

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#### References

Kromus S., Camm B., Camm M., Fowler P. and Narodoslawsky M. (2006) The green biorefinery concept – fundamentals and potential. In: Kamm B., Gruber P.R. and Kamm M. (eds.) *Biorefineries – Industrial Processes and Products*. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp. 253–285. McEniry J., O'Keily P., Clipson N.J.W., Forristal P.D. and Doyle E.M. (2006) The microbiological and chemical composition of baled and precision-chop silages on a sample farm in County Meath. *Irish Journal of Agricultural Food and Research* 45, 73–83.

Wachendorf M., Richter F., Fricke T., Graß R. and Neff R. (2009) Utilization of semi-natural grassland through integrated generation of solid fuel and biogas from biomass. I. Effects of hydrothermal conditioning and mechanical dehydration on mass flows of organic and mineral plant compounds, and nutrient balances. *Grass and Forage Science* 64, 132–143.

# Balance of energy production from high-biodiversity grasslands

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#### Abstract

Maintaining biodiversity of species-rich grasslands is strongly dependent on adapted management particularly in terms of harvest date. Traditional use of such grasslands as forage is declining at a European scale, as it is often not cost-effective due to low nutritional value. Thus, alternative options to use the annually accumulating biomass from semi-natural grasslands are needed. Against the background of the increasing importance of renewable energies, energy recovery from high-diversity grassland is considered as an option to combine landscape conservation and energy production in a profitable way.

The present paper shows a comparative assessment of different options of grassland-use for energy recovery. Highest efficiency of energy conversion is obtained by the hay combustion system. The integrated generation of solid fuel and biogas from biomass leads to somewhat smaller net energy yields, whereas it offers an improved energy carrier to be used in combustion systems and is more independent of weather conditions due to the silage-based procedure. Whole-crop digestion by the dry fermentation system only obtains small net energy yields because of the low digestibility of the highly lignified biomass and a lack of potential waste-heat consumers in rural areas.

Keywords: bioenergy, IFBB, life cycle assessment, semi-natural grassland, solid fuel

## Introduction

Semi-natural grasslands strongly depend on the continuation of extensive agricultural practice. However, in many European regions, an increasing abandonment of high-value grasslands has been observed in the last decades as a consequence of the low economic value of grazing and forage production, and recent evaluations of the situation of European high-value grassland have clearly stated the need to strengthen the measures to keep those sites in a favourable status (Anonymous, 2009). Apart from future strategies to make animal-based management of seminatural grasslands more profitable, the use of grassland biomass for energy recovery becomes increasingly relevant against the background of limited fossil energy resources, climate change and increasing competition of food and energy crops on arable land. Compared to biomass from high-yielding and intensively managed sites, semi-natural grasslands are highly diverse in plant and nutrient composition and rich in fibre content due to the delayed cut and, hence, have special demands on the technique used for the conversion into energy carriers. The present study aims at the comprehensive assessment regarding energy fluxes of a technological approach to produce energy from semi-natural meadows following the integrated generation of solid fuel and biogas from biomass (IFBB) (Wachendorf et al., 2009). Besides the assessment of the IFBB system alternative energy systems, dry fermentation and hay combustion have been calculated considering the energy balance.

#### Materials and methods

The study was based on the principles of life cycle assessment methodology after DIN EN ISO 14040 (Anonymous, 2006), providing a comprehensive analysis of all process steps following the cradle-to-grave approach. It evaluates the energy fluxes within the IFBB system and compares three energy recovery systems concerning their conversion efficiency: integrated generation of solid fuel and biogas from biomass (IFBB), dry fermentation (DF) and hay combustion (HC). The assessment is based on a semi-natural grassland feedstock with a gross yield of 3.8 Mg DM ha<sup>-1</sup> (Hensgen *et al.*, 2011). Within the IFBB procedure the biomass is conserved by ensiling. The silage is processed by hydro-thermal conditioning and mechanical separation, whereas the resulting solid is used as fuel with improved combustion characteristics, and the fluid is converted to biogas with subsequent use by a combined heat and power plant. The DF system is also based on silage which is subjected to whole-crop digestion that takes place in a batch process. The biogas is used by a combined heat and power plant, and 50% of the waste heat is exported. Within the HC system, the biomass is dried on the field and afterwards used for thermal conversion.

#### **Results and discussion**

Hydro-thermal conditioning and separation of the grassland biomass results in a solid fuel with a heat output corresponding to 55% of the gross energy yield. The outcomes of press fluid digestion are completely used to cover internal heat and electrical energy demands (Figure 1).



Figure 1. Energy fluxes within the grassland conversion following the integrated generation of solid fuel and biogas from biomass (primary energy demand disregarded). Numbers in brackets show the percentage of the gross energy yield

Area-related net energy balances show highest energy outputs for the IFBB system by heat and electricity production. However, energy inputs are also highest mainly caused by the heat drying demand for the press cake. The highest net energy yield is obtained by the hay combustion system due to its comparatively low internal energy demand. Low degradability of the silage, as well as insufficient use of the waste heat, leads to low outputs within the dry fermentation system. Transport distances particularly play a role for the wet conservation systems such as IFBB and DF (Figure 2). They account for only a small part of the overall energy inputs, although there is an increase of the diesel input, which varied from 1 to 30 km distance of 106.2, 121.3 and 32.1% for IFBB, DF and HC, respectively.



Figure 2. Net energy balance of the integrated generation of solid fuel and biogas from biomass (IFBB), dry fermentation and hay combustion at varying transport distances

#### Conclusions

Highest efficiency of energy conversion is reached by the hay combustion system. Due to technical constraints when using untreated grassland biomass for combustion and weather risks of field drying conservation, the IFBB approach promises to be an alternative as the net energy yields are only somewhat smaller.

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#### References

Anonymous (2006) DIN EN ISO 14040. Environmental management – Life cycle assessment – Principles and framework. DIN Deutsches Institut für Normung (DIN German Institute for Standardization). Berlin, Germany.

Anonymous (2009) Composite report on the conservation status of habitat types and species as required under article 17 of the Habitats Directive. Commission of the European Communities, Brussels, Belgium. Hensgen F, Bühle L. and Wachendorf M. (2011) Assessment of productivity of semi-natural grasslands under different fertilisation and cutting treatments. *Grassland Science in Europe* 16, 550–552.

Wachendorf M., Richter F., Fricke T., Graß R. and Neff R. (2009) Utilization of semi-natural grassland through integrated generation of solid fuel and biogas from biomass. I. Effects of hydrothermal conditioning and mechanical dehydration on mass flows of organic and mineral plant compounds, and nutrient balances. *Grass and Forage Science* 64, 132–143.

# Possibilities of biomass production within growing sainfoin (*Onobrychis sativa*) for seed

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## Abstract

Sainfoin (*Onobrychis sativa* Lam.) can withstand drought and modest soil conditions, and can be grown in areas that are not suitable for most productive legumes. However, seeding rate for sainfoin is very high, thereby increasing production costs. Under the Bosnian climatic conditions, seed production is more suitable if it is performed at the second vegetative cycle (second cut), due to the higher number of sunny days and good possibility for both pollination and seed setting. Therefore, the first cut may be used for production of roughage and thus reduce the cost of the produced seed. The aim of this paper was to determine possibilities for producing biomass within sainfoin seed production. Different seeding rates (60 and 80 kg ha<sup>-1</sup>), row spacing (20, 40 cm) and phosphorus application (0 vs. 80 kg P ha<sup>-1</sup>) were tested. Results of three years of investigations suggest that the significant yield of biomass (2.3 to 11.5 t ha<sup>-1</sup> DM) can be obtained within seed production. There were positive effects of using 20-cm row spacing on biomass yield.

Keywords: sainfoin, seeding rate, row spacing, biomass yield

## Introduction

Sainfoin (*Onobrychis sativa* Lam.), also known as holy grass or holy hay, is a deep-rooted and very drought-resistant forage legume. It is also winter hardy. Condensed tannins present in sainfoin have been shown to confer anthelmintic properties, increase protein utilisation and prevent bloating. They may also have the potential to reduce greenhouse-gas emissions (Hayot Carbonero *et al.*, 2011). Sainfoin is of high feeding value, with crude protein contents of 10.6–25.2%, depending on row spacing and stage of plant growth. Vasilev and Vasileva recorded sainfoin protein yield of 1.35 t per year.

These characteristics are the reason for new interest in sainfoin. But its high seeding rate makes establishing of sainfoin stands rather expensive. In order to decrease the cost of produced seed, the first cut may also be used for forage production due to the biological feature of sainfoin that it produces a large amount of biomass in the spring. The first cut often yields greater amounts of biomass than alfalfa (Baldridge and Lohmiller, 1990). Under the climatic conditions occurring in Bosnia, seed production is more suitable if harvested from the second growth (2<sup>nd</sup> cut), due to the higher number of sunny days and a good possibility for pollination and seed setting. Depending on growing conditions, sainfoin dry matter yield may range between 7 and 15 t DM ha<sup>-1</sup>. In the first growing year (Zatko, 1972) green mass yield of sainfoin can reach 74.39 t ha<sup>-1</sup>. The objective of this paper was to evaluate the possibility of producing biomass from sainfoin grown for seed.

## Materials and methods

The field experiment (2006–2009) was carried out in Butmir, in the vicinity of Sarajevo (518 m a.s.l.) with an average annual precipitation of 902 mm and an average annual temperature of 9.5°C. Soil of the experimental field is loamy-clay, characterised by pH (water) of 5.6; P was 11 mg, K was 14.6 mg (determined by using amonium lactat-acetic extraction) and N was 160 mg, each in 100 g of dry soil. Nitrogen was determined by the Kjeltec method. We used a randomised complete block design with four replications and plot size of 3.2 m<sup>2</sup>. The experiment was set up in spring 2005, with the following variants: two row spacings (20 and 40 cm), two seeding rates (60 and 80 kg ha<sup>-1</sup>) and two levels of P fertilisation (0.80 kg ha<sup>-1</sup>). The fertilizer was applied early in the spring.

The sward was harvested at the beginning of full flowering time. Biomass yield was measured only from the first cut in years 2006 to 2008, whilst the second cut was used for seed production. Results were subjected to ANOVA and using the F test.

#### **Results and discussion**

Results showed considerable yields of dry biomass in the first growth of *Onobrychis sativa bifera* (the so-called 'two-cut' sainfoin). Depending on treatment and year, the dry matter yield ranged from 2.33 to 11.79 t ha<sup>-1</sup> (Table 1). The highest annual DM yield was achieved in the third year (2008) and the lowest in 2006. Low yield in 2006 could be due to lower temperatures during springtime, compared with the following years, and to insufficiently established plants. However, seeding rate and P fertilisation did not impact significantly on biomass yield within seed production, although higher seeding rate and P fertiliser showed small positive effects on biomass yield at the lower row spacing. On the other hand, row spacing of 20 cm had positive influence (significant in 2007) on biomass yield, for both seeding rates and P fertilizer treatments. Average biomass dry matter yield for the three years of the experiment ranged from 5.46 (without P fertiliser and 80 kg seed ha<sup>-1</sup>) to 9.61 t ha<sup>-1</sup> (without P fertiliser, 20 cm row spacing and 60 kg seed ha<sup>-1</sup>).

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Var	riants	Dry matter yield (t ha <sup>-1</sup> )						
Seeding rate (kg ha <sup>-1</sup> )	Row spacing (cm)	2006	2007	2008	Average			
60	20	2.95	6.62*	11.15	9.61			
60	40	2.79	3.74	9.90	5.47			
80	20	2.33	6.00*	10.51	6.28			
80	40	3.67	4.44	8.40	5.50			
P-Fertilizer (kg ha <sup>-1</sup> )	Seeding rate (kg ha <sup>-1</sup> )	Dry matter yield (t ha <sup>-1</sup> )						
0	60	2.95	6.62	11.15	9.61			
0	80	2.33	6.00	10.51	5.46			
80	60	3.03	5.46	9.55	6.01			
80	80	3.26	5.80	11.79	6.95			
P-Fertilizer (kg ha <sup>-1</sup> )	Row spacing (kg ha-1)		Dry matter y	vield (t ha-1)				
0	20	2.95	6.62*	11.15	9.61			
0	40	2.88	3.74	9.90	5.51			
80	20	3.03	5.46*	9.55	6.01			
80	40	3.24	4.57	9.52	5.78			

Table 1. Effect of seeding rate, row spacing and P-fertilizer on dry biomass yield (t ha-1)

Chemical analysis of biomass (2007 and 2008) showed slightly higher protein content in biomass from the 20 cm row spacing than the 40 cm spacing (13.67% and 11.05% compared to 13.42% and 11.05%, respectively). This result could be expected bearing in mind that stems in a wider row spacing are thicker with more cellulose and less protein. Simple

calculation indicates that considerable protein yield can be obtained from the first-cut biomass (318 to 1524 kg ha<sup>-1</sup>). These results suggest that the contribution of forage production to the total income of a seed production system could be respectable and valuable. However, a more precise answer on the contribution of biomass production to the decreasing cost of seed production has to be done by calculations that take account of all inputs and outputs.

#### Conclusions

Results from this study indicate a good possibility for producing solid dry biomass yield by growing sainfoin for seed. However, biomass yield primarily depends on row spacing and weather conditions. There was less effect on biomass yield shown by seeding rate and P fertilisation. Results also indicate that there is a possibility to decrease the seed production cost.

#### References

Baldridge D.E. and Lohmiller R.G (1990) *Montana Interagency Plant Materials Handbook*. Montana State University, Bozeman, MT. 337 pp.

Hayot Carbonero C., Mueller-Harvey I., Brown T.A. and Smith L. (2011) Sainfoin (*Onobrychis viciifolia*): a beneficial forage legume. *Plant Genetic Resources* 9, 70–85.

Vasilev E. and Vasileva V. (2006) Botanical composition and crude protein content of sainfoin swards under a frequent cutting regime. *Quality Legume-Based Forage System for Contrasting Environments*. Proceedings of the Final Meeting 30<sup>th</sup> August to 3<sup>rd</sup> September 2006. Gumpenstein, Austria, pp. 235–238.

Zatko J. (1972) Vpliv terminu sejbu na urody zelenej hmoty včenca vikolisteha (*Onobrychis viciifolia ssp. sativa* Lam). Tahll. Vedecke prace. 10. VURV. Pieštany.

# Biomass yield and composition from semi-extensively cultivated perennial fodder grasses

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## Abstract

Which perennial fodder grass species is most suitable for energy production from extensive permanent grassland under low fertilizer nitrogen (N) application and low cutting frequency? As part of an experiment comparing annual, perennial and woody candidate biomass crops, four perennial fodder grasses (perennial ryegrass (Lolium perenne L.), timothy (Phleum pratense L.), tall fescue (Festuca arundinacea Schreb.), cocksfoot (Dactylis glomerata L.)) and a mixture of timothy and red (Trifolium pratense L.) and white clover (Trifolium repens L.) were sown in 2007 on a sandy soil in Melle (Flanders) in a split-plot trial with two levels of N application (250 and 150 kg N ha<sup>-1</sup> y<sup>-1</sup>). The plots were cut 3 times a year (in May, July and September) in 2008, 2009 and 2010. We determined the dry matter (DM) yield, the contents of crude protein (CP), water soluble carbohydrates (WSC), neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) and the organic matter digestibility (OMD). The average dry matter yield amounted to 17.2 Mg ha<sup>-1</sup> y<sup>-1</sup> at 250 kg N, and to 14.4 Mg ha<sup>-1</sup>  $v^{-1}$  at 150 kg N. Almost half of the yield was harvested in the first cut. Timothy was the highest yielding species at 250 kg N (19.4 Mg ha<sup>-1</sup> y<sup>-1</sup>) and the mixture of timothy with clover was the highest at 150 kg N (18.2 Mg ha<sup>-1</sup> y<sup>-1</sup>). Perennial ryegrass had the lowest DM yield. Although the higher sugar content and digestibility of the ryegrass, also its area-specific digestible organic matter yield was significantly lower than that of the more fibre-rich species timothy and tall fescue and the timothy-clover mixture at the low N application rate.

Keywords: biomass yield, perennial ryegrass, timothy, tall fescue, cocksfoot

## Introduction

In Flanders (Belgium) perennial ryegrass is the main species used in intensive permanent grassland for feeding ruminants. Permanent grassland may also have some advantages as a feedstock for biogas production. It has a low annual establishment cost, continuous ground cover and flexibility in use. Extensive grassland on marginal soils may be eligible for energy production. Extensive utilization implies low nitrogen fertilizer application and low cutting frequency. Which perennial fodder grass species are the most suitable for biomass production for energy?

## Materials and methods

As part of an experiment comparing annual, perennial and woody candidate biomass crops, the perennial fodder grasses: perennial ryegrass (an early cv. Rebecca and intermediate cv. Plenty), timothy (cv. Comer), tall fescue (cv. Barolex), cocksfoot (cv. Cristobal). and a mixture of timothy and red (cv. Merviot) and white clover (cv. Merwi) were sown in 2007 on a poor sandy soil in Melle (Flanders) in a split-plot trial in 3 replicates with two levels of nitrogen application (250 and 150 kg N ha<sup>-1</sup> y<sup>-1</sup>). The plots (8.1 m<sup>2</sup>) were cut 3 times a year (mid-May, mid-July and mid-September) in 2008, 2009 and 2010, using a Haldrup forage plot harvester equipped with chopper for automatic sampling. We determined dry matter (DM) yield, contents of CP, WSC, NDF, ADF, ADL and *in vitro* OMD (De Boever *et al.*, 1988).

#### **Results and discussion**

The dry matter yield was significantly (P < 0.001) affected by species, N application level, year and cut. As the species × year interaction was not significant, the DM yield is shown in Figure 1 as an average over the 3 harvest years. The average DM yield amounted to 17.2 Mg ha<sup>-1</sup> y<sup>-1</sup> at 250 kg N and to 14.4 Mg ha<sup>-1</sup> y<sup>-1</sup> at 150 kg N. Almost half of the yield (47 %) was harvested in the first cut. Timothy was the species with the highest annual yield at 250 kg N (19.4 Mg ha<sup>-1</sup>). At 150 kg N, the mixture of timothy with clover yielded the most (18.2 Mg ha<sup>-1</sup>). Perennial ryegrass had the lowest DM yield.

The composition of the dry matter is presented in Table 1 as an average of the weighted annual means over the 3 harvest years. Except for the mixture of timothy and clover, the contents of CP, NDF, ADF and ADL were lower, and the WSC content and the digestibility higher, at the low N application rate than at the high N application rate. Perennial ryegrass had the highest WSC content and digestibility. In this respect the intermediate variety was better than the early one. Cocksfoot and timothy had the highest NDF and ADF content. The mixture of timothy with clover had the highest crude protein content but also the highest lignin content.

As an estimation of the area-specific methane yield we have calculated the annual yield of the digestible organic matter (DOM) of the fodder grasses (Table 2). At the 250 N rate, the grass species did not differ significantly, although timothy yielded more than 1 Mg ha<sup>-1</sup> y<sup>-1</sup> more DOM than the perennial ryegrasses. At the 150 N application rate the timothy-clover mixture yielded the most DOM. At this N rate the DOM yield of the perennial ryegrasses was significantly lower than that of timothy, tall fescue and the mixture. These findings are similar to the conclusion of Prochnow *et al.* (2009) who stated that the area-specific methane yield depends on the biomass yield rather than on the species-specific composition.

Table 1. Mean annual content (in g kg <sup>-1</sup> DM) of crude protein (CP), water soluble carbohydrates
(WSC), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin (ADL) and organic mat-
ter digestibility (in %) of early (Lp e) and intermediate (Lp int) heading perennial ryegrass, timothy
(Pp), tall fescue (Fa), cocksfoot (Dg) and a mixture of timothy with clover (Pp+T) at nitrogen ap-
plication rates of 150 and 250 kg ha <sup>-1</sup> y <sup>-1</sup>

Space				150N						250N		
spec.	CP	WSC	NDF	ADF	ADL	OMD	CP	WSC	NDF	ADF	ADL	OMD
Lp e	88	213	538	269	24	72.9	99	176	561	286	26	71.4
Lp int	85	226	523	263	23	75.2	97	189	541	277	24	74.1
Рр	87	141	598	323	36	64.8	102	100	628	343	39	61.7
Fa	82	191	554	283	23	68.3	96	145	590	306	26	65.6
Dg	86	114	626	339	36	65.5	104	87	642	348	39	63.8
Pp+T	118	81	602	349	50	61.8	118	88	615	339	42	62.4
LSD5%	6	17	15	9	2	1.6	9	15	10	8	2	1.4

Table 2. Annual yield of digestible organic matter (average of years 2008, 2009, 2010) of fodder grass species at nitrogen application rates of 150 and 250 kg ha<sup>-1</sup> y<sup>-1</sup> (figures followed by the same letter in the same column are not significantly different by Duncan's multiple range test, P < 0.05)

Species	150N		250N	
Early perennial ryegrass	8165	cd	9797	а
Intermediate perennial ryegrass	7431	d	9898	а
Timothy	9475	ab	11015	а
Tall fescue	9385	ab	10682	а
Cocksfoot	8833	bc	10843	а
Timothy + clover	10404	а	10531	а



Figure 1. Dry matter yield (average of years 2008, 2009 and 2010) of cuts in May (black), July (white) and September (grey) of early (Lp e) and intermediate (Lp int) heading perennial ryegrass, timothy (Pp), tall fescue (Fa), cocksfoot (Dg) and a mixture of timothy with clover (Pp+T) at nitrogen application rates of 150 (left) and 250 kg ha<sup>-1</sup> y<sup>-1</sup> (right)

#### Conclusions

The annual biomass yield of extensively managed perennial ryegrass is lower than that of timothy, tall fescue and cocksfoot. The higher sugar content and digestibility of the ryegrasses, and also their area-specific DOM yield, is significantly lower than that of timothy and tall fescue at an N application rate of 150 kg ha<sup>-1</sup> y<sup>-1</sup>. At this N rate, the highest DOM yield was from the timothy-clover mixture. At the 250 N rate, timothy had the highest DOM yield but the differences in DOM yield between species were not significant. Especially timothy, and to a lesser extent tall fescue and cocksfoot, outyielded perennial ryegrass in terms of biomass production for energy under extensive management.

#### References

De Boever J., Cottyn B., Andries J., Buysse F. and Vanacker J. (1988) The use of a cellulase technique to predict digestibility, metabolizable and net energy of forages. *Animal Feed Science and Technology* 19, 247–260.

Prochnow A., Heiermann M., Plöchl M., Linke B., Idler C., Amon T. and Hobbs P. (2009) Bioenergy from permanent grassland – a review: 1. Biogas. *Bioresource Technology* 100, 4931–4944.

# Biomass of annual forage crops for biogas production

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## Abstract

In Merelbeke (Flanders) a monoculture of maize (+ rye as a catch crop) and a 3-year crop rotation of maize, Italian ryegrass (IR, *Lolium multiflorum*), 1 cut IR + sorghum (+ rye as a catch crop) were compared in terms of biomass production (organic matter yield) (OM) for methane production. Two N-fertilizer regimes were conducted for the annual crops: a moderate and extensive N-regime (i.e. moderate  $\times$  0.6). During a period of 3 years there was no significant difference in OM yield between maize in rotation, maize in monoculture and the combination of 1 cut IR + sorghum at the two N-fertilisation levels. The average yield of 3 years of a monoculture maize (16.6 t OM per ha) was considerably higher than the average yield of a 3-year rotation maize – Italian ryegrass – sorghum (15.2 t OM per ha). The ranking of the individual energy crops in terms of OM production per ha was the same for the moderate and the extensive N fertilization level: maize in rotation > 1 cut IR + sorghum > maize in monoculture > Italian ryegrass. The substrate-specific methane yields of the crops involved in this experiment are comparable, and they have less influence on the methane yield per ha than the area-specific methane yield.

Keywords: biomass yield, composition, maize, sorghum, Italian ryegrass

## Introduction

Maize is the most important energy crop grown for co-digestion in Flanders (5000 ha). In terms of a sustainable agriculture, maize monocultures should be avoided and other energy crops such as Italian ryegrass (IR) and sorghum should be integrated into a crop rotation. Farmers are experienced in growing IR for 1 cut in spring before sowing maize or as a crop for a full year harvest. Sorghum (mainly hybrids *S. bicolor x S. bicolor, S. bicolor x S. sudanense*) can be introduced as a potential crop for biomass production because this C4 plant can produce a large amount of biomass in a short period and has a higher water use-efficiency than maize. Therefore it can be sown as a second crop in combination with one cut of IR, harvested in period 10–25 May. As a result, a maize monoculture, followed by rye as a green cover crop during the winter period is compared with a 3-year rotation of maize – IR – one cut IR + sorghum (+ rye as a winter cover crop). This annual crop production system is part of an experiment comparing annual, perennial and woody candidate biomass crops.

## Materials and methods

The experiment started with an introduction year in 2007, including sowing of IR in autumn to have all the treatments available in the following year. Two cultivars of maize, IR and sorghum (Table 1) were sown in 2007–2010 on a poor sandy soil in Melle (Flanders) in a split-plot trial with three replicates. Two levels of nitrogen fertilization were applied: 150, 250 and  $55+120 \text{ kg N ha}^{-1} \text{ y}^{-1}$  as a moderate level for maize, IR, one cut IR+sorghum, respectively and 60% of this per crop as a low fertilization level. The sowing and harvesting schedule is summarized in Table 1.

		cultivar				sowing date			harvest		
	2008	2009	2010	2008	2009	2010	2008	2009	2010		
maize	Atletico	Atletico	Atletico	7/5	22/4	20/4	2/10	11/9	12/10		
	KWS 1393	34B39	34B39	1/3	23/4	29/4	9/4 5/10		15/10		
sorghum	Green Grazer	Green Grazer	Maja	28/5	5/6 <sup>1</sup>	19/05	14/10	29/9	13/10		
	Goliath	Goliath	Zerberus	20/3				7/10			
Italian ryegrass	Melclips	Melclips	Melclips	24/10/07	10/07 17/10/08	08 14/10/09	1 and	1 and	1 and		
	Gemini	Gemini	Gemini	24/10/07			4 cuts	4 cuts	4 cuts		
rye	Jobaro	Jobaro	Jobaro	24/10	17/10		-	-	-		

Table 1. Cultivars, sowing and harvest period in 2008-2010 (Melle)

<sup>1</sup> date of resowing

#### **Results and discussion**

Two criteria are decisive for choosing crop species for biomethanisation (i) the biomass yield per ha and (ii) the specific methane production rate (CH<sub>4</sub> kg<sup>-1</sup> organic dry matter) (CH<sub>4</sub> = litre of gas by 1013 hPa and 0°C).

Biomass yield per ha.

In a period of three years in this experiment there was no significant difference in OM yield between maize in rotation, maize in monoculture and the combination of one cut IR + sorghum at the two N-fertilisation levels (Table 2). Maize in rotation, maize in monoculture and one cut IR + sorghum yield was, on average over the two N-levels, 16.6, 17.8 and 17.04 t OM per ha, respectively. In the first year, the combination of one cut IR + sorghum had significantly the highest yields of 21.1 and 18.1 t OM per ha for the moderate and low N-levels, respectively, but in the two following years maize in rotation yielded more (only significant in 2009).

Table 2. Organic matter yield of maize, Italian ryegrass and sorghum (kg OM ha-1) at two different
N-fertilisation levels in 2008-2010 (Melle). (Figures followed by the same letter in the same row are
not significantly different by the Scheffe method, $P < 0.05$ )

N fortilization	VOOR	maize	maize	Italian ryegrass	IR + sorghum	average crop
	year	monoculture	in rotation (1)	IR (2)	(3)	rotation (1+2+3)
Moderate	2008	18431b	18669b	11829c	6255+14862a	17205
	2009	18792b	20201a	11214c	4889+12310b	16205
	2010	15980b	17460a	9660c	5840+11203a	14721
	average	17734a	18776a	10901b	5661+12792a	16044
	relat.yield	100, 0	105,9	61,5	31,9+72,1	90,5
Low	2008	15856b	15661b	11037c	5146+12958a	14934
	2009	16863b	19007a	9604c	3483+12071b	14722
	2010	13676b	15750a	8209c	3806+11729a	13165
	average	15465a	16806a	9616b	4145+12253a	14273
	relat.yield	100, 0	108,7	62,2	26,8+79,2	92,3
Average		16600	17791	10259	4903+12522	15159
	relat.yield	100,0	107,2	61,8	29,5+76,0	91,3

Sorghum is not commonly cultivated in this region and crop husbandry can certainly be optimized to improve and stabilize OM yield: e.g. cultivar choice, sowing density, plant protection, N-fertilisation, timing of harvest. IR with one cut between 10 and 20 May produced

31% and 25% of the total yield IR+ sorghum at the moderate and low N-levels, respectively. The annual yield of Italian ryegrass, obtained with grass rather low N-fertilisation rates in a system of four cuts per year, was significantly lower than the other crop yields: 10.9 and 9.6 t OM per ha for the moderate and low N-levels. Maize growing in rotation with other energy crops produced significantly more OM per ha than maize in monoculture in the third (2009) and fourth (2010) growing seasons, but the average yield of three years of a monoculture maize (16.6 t OM per ha) is considerably higher than the average yield of a 3-year rotation with IR and sorghum (15.2 t OM per ha). It will be interesting to see if this will be confirmed during the next three-year rotation period. *Specific methane production rates*.

In the evaluation of different energy crops it is important to consider differences in specific methane production per species, and differences between maize and sorghum were rather small. In a study of the economy of energy crops in the Netherlands, van der Voort et al. (2008) found 300 m<sup>3</sup> methane per t OM for silage maize and 290 m<sup>3</sup> methane per t OM for sorghum (Sudan grass). In the experiments of Ghekiere (personal communication) in Flanders, 396 m<sup>3</sup> and 355 m<sup>3</sup> methane per t OM were measured in maize (n = 11) and sorghum (n = 31) respectively. Grasses have a comparable specific methane production rate to maize silage, but a lower yield potential on many sites (Taube et al., 2007). Although grass species may differ with respect to their chemical composition they appear to be characterised by similar specific methane production rates, ranging between 300 and 400 l<sub>N</sub> kg<sup>-1</sup> organic dry matter. It has been proven by several systematic experiments that specific methane yields decrease with advancing stage of vegetation. Particularly, because hemi-cellulose and lignin are difficult to degrade under anaerobic conditions, increasing the crude fibre limits the biogas production potential (Prochnow et al., 2009). Here, in this experiment, IR was cut four times a year spread out over the growing season with a first cut between 20 and 30 April. This guarantees a low lignin content in the grass: an annual average of 2.37% on dry matter (DM) for lignin and 24.47% on DM for hemi-cellulose was measured in this experiment. A management with an early cut and several cuts per year leads to both high substrate-specific and area-specific methane yields (Prochnow et al., 2008).

#### Conclusions

The annual biomass yield of maize in monoculture is higher than that of a three year rotation of maize – Italian ryegrass – sorghum in the first rotation period. The ranking of the individual energy crops in terms of OM production per ha is the same for the moderate and lower N fertilization level: maize in rotation > one cut IR = sorghum > maize in monoculture > Italian ryegrass. The substrate specific methane yield of the crops, involved in this experiment, are comparable has and have less influence than the area-specific methane yield on the methane yield per ha.

#### References

Prochnow A., Heiermann M., Idler C., Linke B., Plöchl M., Amon T., Langeveld H. and Hobbs P. (2008) Biogas yields from grassland. *Grassland Science in Europe* 13, 727–729.

Prochnow A., Heiermann M., Plöchl M., Linke B., Idler C., Amon T. and Hobbs P. (2009) Bioenergy from permanent grassland – a review: 1. Biogas. *Bioresource Technology* 100, 4931–4944.

Taube F., Hermann A. and Poetsch E. (2007) What are consequences of producing energy crops in the European Union for grassland renovation and new forage production systems? *Grassland Science in Europe* 12, 463–471.

van de Voort, Timmer R., van Geel, Runia W. and Corré W. (2008) Economy of energy crops (in Dutch), Wageningen PPO publication 32500608, 79 p.

## Reduction of mineral concentration of semi-natural grassland biomass used as biofuel through mashing and dewatering

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#### Abstract

Semi-natural grasslands are biodiversity hot spots. To preserve the biodiversity found in these habitats they need to be cut regularly followed by removal of biomass. In order to ensure regular cutting and biomass removal, economically beneficial uses for extensive grassland biomass have to be found. Energy production from low-input grasslands has been proposed as a solution; however, the late harvest proposal leads to a material with high lignin and fibre content that causes low degradability in biogas plants. Combustion of these materials also has been shown to be problematic because of the high content of minerals. Within the European-wide PROGRASS project, the use of semi-natural grasslands from three countries - Germany, United Kingdom and Estonia - for energy production was investigated according to the integrated production of solid fuel and biogas from biomass (IFBB). This study investigates the potential of the IFBB system to produce a solid fuel with improved combustion characteristics from 18 semi-natural grassland sites across Europe (Wales, Germany, Estonia) at a prototype scale.

Keywords: biodiversity, productivity, semi-natural grasslands, IFBB

## Introduction

The growing energy demand and the issue of climate change will lead to an increasing interest in renewable energy from biomass. Grasslands have a large biomass potential, as worldwide there are vast areas covered by grasslands. Semi-natural grasslands are strongholds of biodiversity and they are therefore under special protection. The management regime for these areas very often includes a late seasonal harvest causing a high concentration of fibre and low nutritional value, for which reason the resulting material is not suitable for animal nutrition. The integrated generation of solid fuel and biogas from biomass (IFBB; Wachendorf et al., 2009) is a system developed by the University of Kassel that is especially adapted to this fibre-rich material. The system aims to reduce the mineral elements that are detrimental for combustion (N, S, K, Cl, and Mg) through hydrothermal conditioning and subsequent mechanical dehydration. For the successful use of semi-natural grasslands for combustion it is crucial to produce a solid fuel with low contents of these elements to avoid difficulties in the combustion process, like slagging, fouling, corrosion and emissions. It has been shown in previous studies (Richter et al., 2010) that it is possible to significantly reduce the contents of K, Cl, Mg and S by applying the IFBB approach to German semi-natural grassland material on a laboratory scale.

This study investigates, if these previous results can also be achieved with an up-scaled prototype plant and for materials from different European origins.

#### Materials and methods

Eighteen experimental sites, i.e. six sites each in Germany (DE), Wales (UK) and Estonia (EE), were investigated, and each site had three replicates of  $10 \times 10$  m. Harvesting of the grassland biomass was carried out with a finger-bar mower at a cutting height of 5 cm between June and October in 2009 and 2010. Without wilting the biomass was chopped to an average particle length of 5 cm and then compacted and ensiled for at least six weeks. Pretreatment and mechanical dehydration of the silage was carried out using a mobile prototype plant (Bühle et al., 2010). 20 kg of silage per sample were used for mashing at 30°C for 30 minutes at a ratio of silage to fresh water of about 1:8. Subsequent to mashing, the silage was separated by a screw press (type AV, Anhydro Ltd, Kassel, Germany). The conical screw had a pitch of 1:6, a rotational speed of 3 rev min<sup>-1</sup> and a cylindrical screen with a perforation of 1.5 mm. Samples of silage and press cake (PC) were dried at 60°C for 24 h for chemical analysis. DM content was determined by drving at 105°C for 48 h. Silage and PC were analysed for C, H and N using an elemental analyser (Vario MAX CHN Elementar Analysensysteme GmbH, Hanau, Germany). Content of K, Mg, Cl and S were determined by X-ray fluorescence analysis. Statistical analyses were done using the Software R. Analysis of variance with Tukey HSD as a post-hoc test were performed to test for the effect of IFBB treatment on plant compounds in the silage and PC.

#### **Results and discussion**

One-fifth of the dry matter (DM) of the silage is transferred into the press fluid (PF). Elements showed higher mass flows into the PF, which means that the concentration (in % of dry matter) of these elements in the PC is reduced relative to that of the parent biomass. The highest reduction was achieved for K and Cl, which are highly water-soluble elements. In all three investigated regions more than 77% of K and Cl were directed into the PF. Very low mass flows were observed for N and Ca, whilst S showed a medium mass flow of about 50% into the PF.

	DE	UK	EE
	Mass flow [%]	Mass flow [%]	Mass flow [%]
DM	19.42±0.93 ª	19.43±2.06 ª	18.92±1.09 ª
Crude ash	37.93 ±1.58 ª	49.40±2.49 b	47.86±1.65 ab
Ν	30.63±1.17 ª	36.34±2.06 ª	33.95±1.23 a
S	52.27±1.00 ª	43.57±1.81 b	45.77±1.02 b
Κ	80.14±0.73 °	78.97±1.29 ª	77.43±0.69 a
Mg	64.20±0.99 ª	57.33±1.96 b	57.27±0.90 b
Ca	42.60±1.86 °	40.31±2.64 <sup>ab</sup>	33.60±1.39 b
Cl	86.19±0.77 <sup>a</sup>	85.62±1.31 <sup>ab</sup>	82.80±0.60 b
Р	67.73±2.03 °	62.66±2.60 b	68.94±1.50 °

Table 1. Average mass flow of DM, ash and mineral elements of the silage into the PF with standard error of means, for each of three regions of the study

<sup>a/b/c</sup> indicating significant differences between regions.

The investigated areas and regions showed highly varying results for mineral composition of the silage. The silage was rich in mineral elements and not suitable for direct combustion (Figure 1). The average values for N, S and Cl exceeded guideline values (N: <0.6% DM; S: <0.1% DM; Cl: <0.1% DM) given by Obernberger *et al.* (2006). The use of this grassland silage in combustion systems would lead to increased NO<sub>x</sub> and SO<sub>x</sub> emissions, corrosion and ash slagging. Furthermore, there is a risk of dioxin (PCDD) and furan (PCDF) emissions due



Figure 1. Average concentrations N, S, K and Cl in the silage and press cake semi-natural grassland sites in Germany (DE), Wales (UK) and Estonia (EE). All differences between silage and press cake significant (p < 0.05)

to the high Cl content in the fuel. The IFBB system led to a significant reduction of all mineral concentrations. The PC fulfilled the guiding value for Cl concentration and concentrations of S were close to the guiding value, but for the concentration of N it still exceeded the recommended value. Technical measures, like air-staging in the combus-

tion chamber, will have to be used in order to reduce the  $NO_x$  emissions of N-rich biomass fuels (Obernberger *et al.*, 2006).

#### Conclusions

Due to its high variability in elemental composition, semi-natural grassland biomass is not suitable for energy production through direct combustion, as it generally shows high concentrations of Cl, N, K, S and Mg, which would lead to emissions, corrosion and ash melting. The IFBB system improved the quality of the biomass of European grasslands for combustion by reducing mineral contents of the fuel significantly. The IFBB Prototype proved to be successful, as the results for mineral reduction were comparable to earlier laboratory research conducted by Richter *et al.* (2010).

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## References

Bühle L., Donnison I., Heinsoo K., Südekum K.-H. and Wachendorf M. (2010) PROGRASS – A mobile plant to produce solid fuel from grass harvested in the NATURA grassland habitats. *Grassland Science in Europe* 15, 208–210.

Obernberger I., Brunner T. and Bärnthaler G. (2006) Chemical properties of solid biofuels-significance and impact. *Biomass and Bioenergy* 30, 973–982.

Richter F., Fricke T. and Wachendorf M. (2010) Utilization of semi-natural grassland through integrated generation of solid fuel and biogas from biomass. III. Effects of hydrothermal conditioning and mechanical dehydration on solid fuel properties and on energy and green house gas balances. *Grass and Forage Science* 65, 185–199.

Wachendorf M., Richter F., Fricke T., Graß R. and Neff R. (2009) Utilization of semi-natural grassland through integrated generation of solid fuel and biogas from biomass. I. Effects of hydrothermal conditioning and mechanical dehydration on mass flows of organic and mineral plant compounds and nutrient balances. *Grass and Forage Science* 64, 132–143.

# Can specific methane yield be enhanced by co-fermenting different crop substrates?

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## Abstract

The concept of co-digestion is well established as an option to increase yield of anaerobic digestion in waste treatment. What is not yet clear is whether crop substrates would have synergistic or antagonistic effects on specific methane yield when digested with other crops. Comparing single crop digestions of maize, whole-crop wheat, first-cut grass, and second-cut grass with various binary or tertiary mixtures, synergistic interactions up to 4.9% as well as adverse effects up to 9.4% were found, which, however, could not clearly be deduced.

Keywords: anaerobic digestion, substrate mixture, silage maize, whole-crop wheat, perennial ryegrass

#### Introduction

Energy cropping for biogas production has become an important production branch for many farms in Germany. Currently, maize is the main crop substrate grown for fermentation. Potential negative effects of an increasing maize acreage, however, raise the interest in biogas crop rotations. In addition, recent research provides evidence that mono-digestion of maize can result in process instability due to imbalanced macro- and micronutrient contents (Bruni *et al.*, 2010; Demirel and Scherer, 2011). As is well known, crop dry matter yield is the main driver of methane hectare yield  $[m_N^3 CH_4 ha^{-1}]$ . Variation in specific methane yield  $[l_N CH_4 kg oDM^{-1}]$  due to crop species, developmental stage, or fertilizer supply contributes to a smaller degree, but should not be overlooked. Whilst synergistic and antagonistic effects of co-digestion are known from anaerobic waste treatment, there are few available data concerning the co-fermentation effect by systematically analysing the specific methane yield (SMY) of various fresh crop substrates fermented, either separately or in defined mixtures.

## Materials and methods

Crop samples of silage maize, whole-crop wheat, first-cut grass (*Lolium perenne*), and second-cut grass (*L. perenne*) were obtained from a field experiment, where the impact of cropping system, N fertilizer type, and N amount on yield performance and environmental effects was investigated at a coastal marsh site in northern Germany. All samples comprised pooled samples from 4 field replicates of optimally fertilised treatments harvested at silage maturity (maize, wheat: dough stage, first-cut grass: heading) in 2010. Samples were dried (58°C) and milled (1 mm), and fermented separately, in binary mixtures (0–100%) of maize-1<sup>st</sup>cut grass, maize-2<sup>nd</sup>cut grass, maize-wheat, wheat-1<sup>st</sup>cut grass, and in tertiary mixtures

of maize-wheat-1<sup>st</sup>cut grass and maize-wheat-2<sup>nd</sup>cut grass. The specific methane yield was determined in three replicates by using the Hohenheim biogas yield test, conducted in compliance with the German Standard Procedure VDI 4630 (VDI, 2006) and Helffrich and Oechsner (2003). About 300 mg sample was fermented with 30 ml inoculum (sewage sludge) for 28 days at 38°C. The ratio of substrate to inoculum was equal to or less than 0.5 (oDM basis). Each run included three references of each, a pure inoculum and a microcrystalline cellulose sample. An Advanced Gasmitter (AGM 10, Sensors Europe) was employed for gas analysis, and SMY was adjusted to norm conditions. Crop substrate characteristics were quantified in terms of the contents of ash, nitrogen, crude fat, ADF, NDF, watersoluble carbohydrates, starch, enzyme soluble organic matter (ELOS) as well as by energy concentration; see Table 1. Multiple contrast tests (Dilba *et al.*, 2006) were conducted to compare the ratios of actual versus theoretical SMY, using R.

	Maize	Wheat	Grass, 1st cut	Grass, 2 <sup>nd</sup> cut
Dry matter content	304.4	416.8	238.2	291.7
Ash	44.0	91.0	83.0	120.0
Nitrogen	12.6	15.2	18.6	26.4
Crude fat	21.0	17.0	32.0	31.0
ADF	234.0	308.0	200.0	228.0
NDF	478.0	502.0	450.0	518.0
Water-soluble carbohydrates	n.a.	n.a.	186.0	92.0
Starch	282.0	264.0	n.a.	n.a.
ELOS*	674.0	594.0	752.0	655.0
Energy concentration (ME kg <sup>-1</sup> DM)	10.4	9.3	11.3	10.3

Table 1. Characteristics of the crop substrates (g kg<sup>-1</sup> DM)

\* Enzyme soluble organic matter; n.a. not available.

#### **Results and discussion**

Observed SMY varied between 341 and 393  $l_{N}$  CH<sub>4</sub> kg<sup>-1</sup> oDM (Table 2), which is within the range reported in the literature. Fermentation of pure maize and grass samples resulted in similar SMY values, and maize-grass mixtures did not show significant differences between theoretical and actual SMY. Antagonistic and synergistic effects of co-digestion ranging between -9.4 and 4.8% were only detected for mixtures including whole-crop wheat. For 2 out of 5 maize-wheat mixtures, the measured SMY significantly exceeded the values that would be expected if the SMY were purely additive, whilst for 4 out of 10 maize-wheatgrass mixtures a significantly adverse effect on SMY was detected. Methane content, which varied between 55.2 and 59.5%, tended to be lower for mixtures having a negative difference between theoretical and actual SMY. Mukengele et al. (2006) reported up to 8% higher SMY for binary mixtures of silages of maize, clover-grass, fodder beet, and whole-crop rye. A study by Amon et al. (2007) on co-digestion of various crop silages and pig slurry found relative differences between theoretical and actual SMY to vary between -2.4 and 48.5%. Causes for the observed effects, however, are not clear-cut. Binary maize-wheat mixtures 4 and 5 showed improved SMY with increasing wheat proportions. In accordance, tertiary mixtures 24 and 25, which were characterized by having low wheat and grass proportions, showed antagonistic effects. This might be due to effects on N content and C/N ratio, which need to be in an optimum range for a stable anaerobic digestion (Braun, 1982). Trace element content, which is often considered a limiting factor in mono-digestion (Demirel and Scherer, 2011), is regarded as relevant only for long-term fermentation. The adverse effect found for mixture 23 could be attributed to a high protein content resulting in ammonia inhibition. However, we would then have expected similar effects for maize-grass and wheat-grass binary mixtures.

	Sam	ple compos	sition (% o	f DM)				
Mixture	Maize	Wheat	1 <sup>st</sup> cut grass	2nd cut grass	Theoretical meth- ane yield (1 <sub>N</sub> kg <sup>-1</sup> oDM)	Actual methane yield (l <sub>N</sub> kg <sup>-1</sup> oDM)	Relative difference (% of theor.)	Р
	100					384.2		
		100				345.7		
			100			386.6		
				100		372.2		
1	85	15			378.4	377.0	-0.4	0.999
2	75	25			374.5	376.7	0.6	0.996
3	50	50			364.9	362.4	-0.7	0.992
4	25	75			355.3	369.8	4.1	0.050
5	15	85			351.4	368.1	4.8	0.022
6	85		15		384.5	389.5	1.3	0.997
7	75		25		384.8	387.3	0.7	0.998
8	50		50		385.4	379.8	-1.5	0.849
9	25		75		386.0	384.4	-0.4	0.998
10	15		85		386.3	388.9	0.7	0.997
11	75			25	381.2	375.0	-1.6	0.754
12	50			50	378.2	377.9	-0.1	1.000
13	25			75	375.2	384.1	2.4	0.497
14		85	15		351.8	361.9	2.9	0.521
15		75	25		355.9	373.2	4.9	0.164
16		50	50		366.2	358.7	-2.0	0.752
17		25	75		376.4	393.0	4.4	0.195
18		15	85		380.5	375.9	-1.2	0.962
19	10	80	10		353.6	352.0	-0.5	1.000
20	20	60	20		361.6	356.6	-1.4	0.958
21	33	33	33		368.4	361.0	-2.0	0.747
22	20	20	60		378.0	364.3	-3.6	0.137
23	10	10	80		382.3	346.3	-9.4	0.001
24	60	20	20		377.0	362.0	-4.0	0.091
25	80	10	10		380.6	360.2	-5.4	0.047
26	10	10		80	370.7	341.1	-8.0	0.001
27	20	20		60	369.3	355.6	-3.7	0.076
28	33	33		33	363.7	343.3	-5.6	0.004

Table 2. Effect of varying crop substrate ratios on theoretical and actual specific methane yield  $(l_{\lambda} kg^{-1} CH_{A} oDM)$ ; *P*-value obtained by multiple contrast tests

#### Conclusions

Both, synergistic and antagonistic effects, were detected when co-digesting crop substrates. Further analyses, including batch or continuous tests with longer incubation time, are required to elucidate the underlying processes.

#### References

Amon T., Machmüller A., Kryvoruchko V., Milovanovic D., Hrbek R., Eder M.W. and Stürmer B. (2007) Optimierung der Methanausbeute aus Zuckerrüben, Silomais, Körnermais, Sonnenblumen, Ackerfutter, Getreide, Wirtschaftsdünger und Rohglyzerin unter den Standortbedingungen der Steiermark. *Forschungsprojekt* Nr. 1421. Endbericht.

Braun R. (1982) Biogas-Methangärung organischer Abfallstoffe. Springer, Wien.

Bruni E., Jensen A.P., Pedersen E.S. and Angelidaki I. (2010) Anaerobic digestion of maize focusing on variety, harvest time and pretreatment. *Applied Energy* 87, 2212–2217.

Demirel B. and Scherer P. (2011) Trace elements requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass and Bioenergy* 35, 992–998.

Dilba G., Bretz F. and Guiard V. (2006) Simultaneous confidence sets and confidence intervals for multiple ratios. *Journal of Statistical Planning and Inference* 136, 2640–2658.

Helffrich D. and Oechsner H. (2003). The Hohenheim Biogas Yield Test. Landtechnik 58, 148-149.

Mukengele M., Brule M. and Oechsner H. (2006) Einfluss der Substratmischung aus Energiepflanzen auf Abbaukinetik und Methanertrag. *Landtechnik* 61, 26–27.

VDI (2006) VDI 4630: Vergärung organischer Stoffe – Substratcharakterisierung, Probennahme, Stoffdatenerhebung, Gärversuche. Berlin, Beuth Verlag GmbH.

# The variation of structural biopolymers in the biomass of perennial grasses used for biogas

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## Abstract

Biomass of grasses as raw material for biogas production is rich in easily biodegradable components such as lipids, sugars, proteins and starches. On the other hand, it contains more than 60 percent of other fractions – hemicellulose, cellulose and lignin whose microorganisms are more difficult to degrade. The concentration of structural biopolymers in the biomass depends on grass species. The aim of the present study was to evaluate the variation of the concentration of hemicellulose, cellulose and lignin in the biomass of perennial grasses grown under different conditions. Two (three in 2009) or three (four in 2009) cuts per season of tall fescue, cocksfoot and reed canary grass were managed with two nitrogen fertilization levels  $N_{90}$  and  $N_{180}$ .

Keywords: perennial grasses, lignin, cellulose, hemicellulose

## Introduction

Production of bioenergy on the basis of anaerobic digestion has become very popular in Europe. Perennial grasses could be selected as promising energy crops for biogas production (Amon *et al.*, 2007; Jasinskas *et al.*, 2008; Prochnow *et al.*, 2009). The factors which determine biogas yield of perennial grasses are biomass yield and quality. Usually, biomass of grasses consists of 20–30% of hemicellulose and about 40% of cellulose. It is difficult for bacteria to decompose these elements, but the usage of pretreatments can help to break down the cellulose and make it more suitable for the digestion of microorganisms (Carvalheiro *et al.*, 2008; Weiß *et al.*, 2010). The higher concentration of these structural biopolymers increases the biogas yield and energy value of anaerobic process (Amon *et al.*, 2007).

On the other hand, the main task in choosing energy crops for biogas production is to select the most productive grass species which have the highest concentration of biodegradable material. The biodegradability is usually described as biomass without lignin. Lignin is a natural biopolymer which reduces the digestibility of cellulose and hemicellulose. Anaerobic bacteria cannot break it down and it cannot be digested. Lignin can act as an inhibitor for the digestion of hemicellulose and cellulose (Casler *et al.*, 2008).

The aim of this study was to evaluate the impact of mineral fertilizers and the maturity stage of perennial grasses on the yield and concentration of structural biopolymers: cellulose, hemicellulose and lignin.

## Materials and methods

Laboratory and field experiments were carried out at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, during 2008–2010. Tall fescue (*Festuca arundinacea*), cocksfoot (*Dactylis glomerata*) and reed canary grass (*Phalaris arundinacea*) were grown in the soil, which contained: organic carbon – 1.61–1.75%, available P 145–224 mg kg<sup>-1</sup> and K – 128–158 mg kg<sup>-1</sup>; soil pH was between 6.7 and 7. N<sub>90</sub> and N<sub>180</sub> nitrogen fertilizer rates were used in the experiments. The swards were cut three (first-cut at heading stage) and four (first-cut at flowering stage) times per season in 2009 and two and three times per season in 2010. The concentration of hemicellulose, cellulose and lignin was determined using the Van Soets methodology for fibre fraction.

#### **Results and discussion**

In our research, the yield of cellulose was influenced by the grass species, cutting frequency and nitrogen fertilization (Figure 1). The highest cellulose yield was produced by the tall fescue swards. The results of the two experimental years suggest that tall fescue produced higher, and reed canary grass produced lower, cellulose yields when the swards' first cut had been taken at flowering time, compared to those at which the first time cut was at heading. The cutting frequency did not have any significant impact on cellulose productivity of cocksfoot. Sward fertilization with 180 kg ha<sup>-1</sup> of nitrogen fertilizer increased the cellulose yield on average, compared to 90 kg ha<sup>-1</sup> nitrogen rate. The effect of fertilizers was also influenced by the weather conditions.



Figure 1. The variation of cellulose yield per hectare of tall fescue, cocksfoot and reed canary grass in the first year of swards use

The results of the two experimental years suggest that tall fescue was characterized by the highest yield of hemicellulose (Figure 2). Tall fescue swards harvested twice (three times in 2019) per season produced significantly higher hemicellulose yield, compared to those harvested three times (four times in 2019) per season. The hemicellulose yield of cocksfoot and reed canary swards did not differ significantly between the 2-cut and 3-cut systems. Fertilization with the higher nitrogen fertilizer rate (180 kg ha<sup>-1</sup>) had a positive effect on most treatments. In our research, the concentration of lignin was influenced by the grass species, sward age and cutting frequency. The concentration of lignin was the lowest in the tall fescue biomass in the first cut taken at heading stage. Worse results were obtained in reed canary biomass,



Figure 2. The variation of hemicellulose yield per hectare of tall fescue, cocksfoot and reed canary grass in the first year of swards use

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where the concentration of lignin varied from 4 to 8% of DM. Later harvesting resulted in a higher concentration of lignin in the biomass of all swards. When the concentration of lignin in biomass is higher than 15% DM, the anaerobic digestion is sharply inhibited (Raclavská *et al.*, 2007). In our research in all swards the concentration of lignin was lower than 10% of dry matter.

			Year 2009				Year 2010		
					Cuts				
		1	2	3	4	1	2	3	
		Ta	all fescue						
First out at boading	$N_{90}$	3.377	2.530	7.280	5.680	2.850	4.190	4.150	
First cut at heading	N <sub>180</sub>	3.310	4.060	7.480	4.900	3.430	7.270	3.670	
	N <sub>90</sub>	4.593	2.590	5.760	_	4.910	3.870	_	
First cut at flowering	$N_{180}^{90}$	4.470	4.930	5.200	-	5.910	4.460	-	
	Cocksfoot								
First cut at heading	N <sub>90</sub>	2.853	4.540	9.430	7.810	3.750	4.670	3.850	
	N <sub>180</sub>	2.960	3.940	9.680	8.420	3.800	7.590	4.390	
First cut at flowering	N <sub>90</sub>	5.340	4.320	6.360	_	5.470	5.080	_	
-	N <sub>180</sub>	4.683	4.240	7.420	-	5.750	5.660	-	
Reed canary grass									
	N <sub>90</sub>	6.210	4.550	7.770	5.120	5.970	7.720	4.290	
First cut at heading	N <sub>180</sub>	6.863	4.210	8.700	7.550	5.820	7.260	4.070	
Einst aut at flammin a	N <sub>90</sub>	5.650	5.220	6.390	—	6.850	6.210	_	
First cut at flowering	N <sub>180</sub>	4.943	4.650	9.460	_	7.360	6.540	_	

Table 1. The concentration of lignin in % of DM in the biomass of tall fescue, cocksfoot and reed canary grass in the first year of swards use

#### Conclusions

In the first year of sward use, tall fescue produced the highest yield of structural biopolymers compared to cocksfoot and reed canary grass. The tall fescue swards first-time cut at flowering had higher yield of cellulose and hemicellulose compared to those with the first-time cut at heading. The cutting frequency did not have any significant effect on the yield of structural biopolymers of cocksfoot and reed canary grass. In all swards, the concentration of lignin was below 10%. The results of the first year of sward use suggest that tall fescue could be a suitable energy crop for biogas production.

#### References

Amon T., Amon B., Kryvoruchko V., Machmuller A., Hopfner-Sixt K., Bodiroza V., Hrbek R., Friedel J., Poutsch E., Wagentristl H., Schreiner M. and Zollitsch W. (2007) Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Bioresource Technology* 98, 3204–3212. Casler M.D., Jung H.G. and Coblentz W.K. (2008) Clonal selection for lignin and etherified ferulates in three perennial grasses. *Crop Science* 48(2), 424–433.

Carvalheiro F., Duarte L.C. and Girio F.M. Hemicellulose biorefineries: a review on biomass pretreatments. *Journal of Scientific and Industrial Research* 67, 849–864.

Jasinskas A., Zaltauskas A., and Kryževičienė A. (2008) The investigation of growing and using of tall perennial grasses as energy crops. *Biomass and Bioenergy* 32(11), 981–987.

Prochnow A., Heiermann M., Plöchl M., Linke B., Idler C., Amon T. and Hobbs P. (2009) Bioenergy from permanent grassland – A Review: 1. Biogas. *Bioresource Technology* 100, 4931–4944.

Raclavská H., Juchelková D., Roubíček V., and Wiltowski T. (2007) *Utilization of Biomass and Mixtures for Gas Production*. International Conference on Engineering Education – ICEE 2007, Coimbra, Portugal. Weiß S., Tauber M., Somitsch W., Meincke R., Müller H., Berg G. and Guebitz G.M. (2010) Enhancement of biogas production by addition of hemicellulolytic bacteria immobilised on activated zeolite. *Water Research* 44(6), 1970–1980.

## The tensile strength of two grass species at two contrasting growth stages and their subsequent reinforcement properties in clay blocks

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## Abstract

This study investigated (a) the tensile strength of two grass species at two contrasting growth stages, and (b) the potential of the fibre-rich press-cake fraction from these grasses to enhance the mechanical properties of clay blocks. Thirty grass plants were sampled from triplicate field plots of each of two grass species, perennial ryegrass and timothy, at two dates (25 May, 22 June) in the primary growth. Stem and leaf sections (9 cm) were sampled from each plant, oven dried and used to determine tensile strength. In addition, representative grass samples from each plot were ensiled in laboratory silos and the resulting silages were separated into nutrient-rich press-juice and fibre-rich press-cake fractions. The separated press-cake fraction was dried and its potential as a fibre-reinforcement in standard pottery clay blocks ( $80 \times 40 \times 40$  mm) was assessed at four inclusion rates (0, 1.5, 3.0 and 4.5% by weight). On average, grass stems had a numerically higher tensile strength than grass leaves, with the stems of timothy being greater (P < 0.01) than those of perennial ryegrass. The compressive strength of clay blocks decreased with increasing press-cake inclusion rate.

Keywords: grass, press-cake, tensile strength, clay blocks, compressive strength

## Introduction

In recent years interest has developed in alternative uses of grass for purposes other than as a herbivore feedstuff (e.g. renewable energy, bio-based materials). In the initial stages of a 'Green Biorefinery' process, plant biomass is separated into a fibre-rich press-cake (PC) and a nutrient-rich press-juice, and these separated fractions are further refined to recover or produce industrial products. For example, Grass (2004) described the potential of the PC fraction for thermal insulation boards. This study investigated the mechanical properties of grasses sampled at two stages of growth and the potential of the separated PC fraction to enhance the mechanical properties (compressive strength) of clay blocks.

## Materials and methods

Thirty grass plants were sampled from triplicate field plots (each 20 m<sup>2</sup>) of each of two grass species, perennial ryegrass (PRG; *Lolium perenne* L. cv. Gandalf) and timothy (*Phleum pratense* L. cv. Erecta) which had fertiliser nitrogen applied at 125 kg N ha<sup>-1</sup> and were harvested at two dates (Harvest 1 = 25 May and Harvest 2 = 22 June 2010) in the primary growth. The phenological stage of development of PRG and timothy was 2.5 and 2.3 at Harvest 1, and 3.5 and 3.3 at Harvest 2, respectively according to Moore *et al.* (1991). A 9 cm stem (1.5–10.5 cm above ground) and leaf (second-most mature leaf; approximately midway between the leaf collar and the leaf tip) section were sampled from each plant and oven-dried at 40°C for 48 h. The tensile strength of grass stem and leaf sections was determined using a Hounsfield H25K-S testing machine (Tinius Olsen Ltd., Surrey, UK). A 10 kN

load cell was used and the loading rate was 10 mm min<sup>-1</sup>. The diameter of the stem, and the width and thickness of the leaf at the point of fracture were determined using a digital callipers (Mitutoyo, Absolute, Kawasaki, Japan). Tensile strength was calculated as the force per cross-sectional area (N mm<sup>-2</sup>) at the point of fracture. Representative samples of PRG, grown in triplicate field plots as described above, were harvested using a Haldrup forage plot harvester (J. Haldrup, Løgstor, Denmark) on two dates (Harvest 1 = 25 May, and Harvest 2 = 22 June 2010), precision-chopped (19 mm nominal chop length) and ensiled in laboratory pipe silos at 15°C. After 100 days ensilage, representative silage samples (n = 3) were subjected to hydrothermal conditioning (3 water: 1 silage at 60°C for 30 minutes, with mixing) prior to mechanical pressing (hydraulic press) at 4.5 MPa to isolate the fibre-rich PC fraction. The PC fractions were oven-dried at 48°C for 48 h. Representative PC samples were included at rates of 0 (i.e. control), 1.5, 3.0 and 4.5% (by weight) to standard terracotta pottery clay made with a water content of 17.5%. For each treatment (i.e. silage replicate  $\times$  fibre inclusion rate), 8 replicate clay blocks were made using prism moulds (80×40×40 mm) and were allowed to cure for 14 days in a controlled environment of 18°C and 55% relative humidity before determining compressive strength using a TRISCAN 50 testing machine (VJ-Tech Ltd., Berkshire, UK). For tensile strength, data were analysed as a split-plot design, with harvest date as the main-plot and species as the sub-plot for both leaf and stem, while for compressive strength, data were analysed as a split-plot design with harvest as the main plot and PC inclusion rate as the sub-plot.

#### Results

There was little difference (P > 0.05) in the tensile strength of leaf sections across the two harvest dates or grass species (Table 1). On average, grass stems had a numerically higher tensile strength compared to grass leaves. In general, the tensile strength of timothy stems was greater (P < 0.01) than that of PRG stems. Although the Harvest2 grass stems (53.9 N mm<sup>-2</sup>) had a numerically higher tensile strength compared to Harvest 1 (43.0 mm<sup>-2</sup>) they were not significantly different (P > 0.05). The addition of the PC fraction at increasing inclusion rates to clay Table 1. Average tensile strength of the leaf and stem sections of perennial ryegrass (PRG) and timothy at two harvest dates (H1 = 25 May and H2 = 22 June 2010) in the primary growth

Harvest	Grass species	Plant part	Tensile strength (N mm <sup>-2</sup> )
H1	PRG	Leaf	19.8
H1	Timothy	Leaf	20.4
H2	PRG	Leaf	16.4
H2	Timothy	Leaf	22.1
H1	PRG	Stem	36.8
H1	Timothy	Stem	49.1
H2	PRG	Stem	42.6
H2	Timothy	Stem	61.2
SEM <sup>1</sup>	2		3.21

<sup>1</sup>Standard error of the mean (for interaction; harvest  $\times$  grass species).

Table 2. Average compressive strength of clay blocks made with press-cake (PC) from two harvest dates (H1 = 25 May and H2 = 22 June 2010) and at different inclusion rates

< J	,	
	PC inclusion rate (%)	Compressive strength (N mm <sup>-2</sup> )
Control	0	2.72
H1	1.5	1.78
H1	3.0	1.04
H1	4.5	0.74
H2	1.5	1.24
H2	3.0	0.86
H2	4.5	0.64
SEM <sup>1</sup>		0.084

<sup>1</sup>Standard error of the mean (for interaction).

blocks resulted in a decrease (P < 0.001) in compressive strength of the blocks compared with the clay control (0% PC inclusion; Table 2). The compressive strength of clay blocks reinforced with PC from the Harvest 1 silage was higher (P < 0.05) at the 3.0 and 4.5% inclusion rates than the PC from the corresponding Harvest 2 silage treatments.

#### Discussion

As expected, the tensile strength of the stem sections was greater than that of the leaf sections and this is related to the higher fibre concentration of grass stems than leaves providing greater mechanical support for aerial shoots. The numerical increase in tensile strength with advancing harvest date reflects the increase in chemical fibre concentration (data not shown) with advancing plant maturity, but this difference was not significant. The average tensile strength measurements for stem sections in this study ranged from 36.8–49.1 N mm<sup>-2</sup> at the early harvest date and 42.6–61.2 N mm<sup>-2</sup> at the later harvest date, and are within the range reported by Jacobs et al. (2011) and Galeder et al., (2008) for stems of tropical grass species (4.4–11.7 N mm<sup>-2</sup> in the elongation stage to 19.0-63.9 N mm<sup>-2</sup> in the anthesis stage) and alfalfa (44 N mm<sup>-2</sup> at 90% dry matter content), respectively. Bouhicha et al. (2005) reported that straw reinforcement of soil blocks up to a 1.5% inclusion rate enhanced the compressive strength of the blocks; however, further increases in the fibre inclusion rate decreased the compressive strength. This differs from the current study where no rate of PC inclusion increased the compressive strength of clay blocks relative to that of the non-reinforced block (i.e. control) and implies that the PC fraction is not a suitable reinforcement for load bearing structures. However, compressive strength at the low inclusion rate (1.5%) was comparable to that of traditional mud bricks (clay, straw and water; 1.2 to 1.8 N mm<sup>-2</sup>; Binici et al., 2007).

#### Conclusions

The grass stems had a higher tensile strength than grass leaves, with stems of timothy being greater in tensile strength than those of PRG. The compressive strength of clay blocks decreased with increasing PC inclusion rate. Further work will investigate the potential of PC inclusion to reduce cracking in soil and mortar specimens.

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## References

Binici H., Aksogan O. and Nuri B.M. (2007) Thermal isolation and mechanical properties of fibre reinforced mud bricks as wall materials. *Construction and Building Materials* 21, 901–906.

Bouhicha M., Aoussi F. and Kenai S. (2005) Performance of composite soil reinforced with barley straw. *Cement and Concrete Composites* 27, 617–621.

Galedar M.N., Jafari A. and Mohtasebia S.S. (2008) Effects of moisture content and level in the crop on the engineering properties of alfalfa stems. *Biosystems Engineering* 101, 2, 199–208.

Grass S. (2004) Utilisation of grass for production of fibres, protein and energy. *Biomass and Agriculture: Sustainability, Markets and Policies*, pp. 169–177, Paris, France, OECD Publication Service.

Jacobs A.A.A., Schepper J.A., Benvenutti M.A., Gordon I.J., Poppi D.P. and Elgersma A. (2011) Tensile fracture properties of seven tropical grasses at different phenological stages. *Grass and Forage Science* 66, 4, 551–559.

Moore K.J., Moser L.E., Vogel K.P., Waller S.S., Johnson B.E. and Pedersen J.F. (1991) Describing and quantifying growth stages of perennial forage grasses. *Agronomy Journal* 83, 1073–1077.

# Anaerobic digestion of grassland biomass: Effects of species richness and functional groups on potential methane yield

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## Abstract

The generation of energy from semi-natural, high diversity grasslands can be an alternative to the abandonment of such sites, which is a noticeable trend in many developed countries. One possible path of converting grassland biomass to energy is through anaerobic digestion of silages. Little is known about the linkages between increasing plant diversity and the effects on energy production. In this study, changes in energy content (substrate-specific methane yield) as well as the respective energy yields (methane yield per hectare), along a well defined diversity gradient (1-60 species) and across different combinations of functional groups (legumes, small herbs, tall herbs and grasses), were investigated. The substrate-specific methane yields were estimated through the concentrations of the Weende constituents and their documented digestibility values. It was found that increasing diversity had a negative effect on energy content, while the energy yield increased due to a strong increase in biomass with increasing diversity. Energy content and yield varied between functional group monocultures and all functional group mixtures.

Keywords: digestibility, diversity, energy, fibre, functional groups, methane

## Introduction

Semi-natural grasslands, originally used for animal nutrition, have many ecosystem functions and are known to be hotspots of biodiversity in rural areas. However, their species diversity is expected to decline. Causes for that loss are changes in land use. In developed countries the fodder quality of cut semi-natural grassland is of decreasing suitability for animals with high milk and meat performance, leaving the farmer with the option to either intensify the grassland management or retreat from it. For some regions in central Europe it was estimated that up to one quarter of permanent grassland will be abandoned in the near future. This leads to a surplus of permanent grassland that requires a management of at least one cut per year if its species richness is to be protected. As the biomass is not suitable for animal nutrition it may be available for energy production. Anaerobic digestion of extensive grassland biomass for biogas production is one conversion technique that can help reduce greenhouse gas emissions and in maintaining biodiversity. The focus of this study was on the linkages between diversity parameters (species richness, functional groups) and energy parameters (substrate-specific methane yield and area specific methane yield). The methane yields presented here were estimated from the biomass characteristics as described by the Weende constituents.

## Materials and methods

In May 2002 an experimental site with semi-natural mesophilic grassland was established in the floodplain of the River Saale (near Jena, Germany, 130 m a.s.l.). Sixty plant species were used to create a gradient in plant species richness (1, 2, 4, 8, 16, 60) and in functional group richness (1, 2, 3, 4). Functional groups were grasses, small herbs, tall herbs and legumes. Eighty-two plots were established on four blocks, the blocks accounting for the differences in soil texture. Sixteen possible combinations of species richness and functional group richness were realized and replicated over the four blocks. The location of the mixtures within each block was fully randomized. Management of the site was two cuts per year (late May and late August) and no application of fertilizer. Plots were weeded twice a year. The experimental setup is described in full detail in Roscher *et al.* (2004).

Aboveground biomass was harvested twice in the years 2008 and 2009 right before the first (end of May) and second cut (end of August). For crude fibre (XF) and crude lipid (XL), 100 of the 164 samples were analysed in the laboratory according to standard methods (Naumann and Bassler, 2004) and then used for near-infrared-calibration. With a near-infrared-spectroscope (XDS Rapid Content Analyser, FOSS NIRSystems Inc., Laurel, USA) all values for the 164 samples were predicted after cross-validation (XF: R2 = 0.96, RPD = 3.68; XL: R2 = 0.74, RPD = 1.70). Crude ash (XA) was determined by loss on ignition at 550°C, whilst crude protein (XP) was calculated from the nitrogen content analysed with an elemental analyser (vario MAX CHN, Elementar Analysensysteme GmbH, Hanau, Germany). Nitrogen-free extract (NfE) was calculated as: NfE = 100 - XA - XF - XL - XP (all in % DM) and the sum of NfE and XF was assumed to represent the fraction of carbohydrates.

To calculate the potential substrate-specific methane  $(CH_{4 sub})$  yield of this heterogeneous dataset, the method of VDI 4630 (2004) was modified to also account for the effects of botanical composition and harvest time in the calculations. The method is based on the concentrations of the Weende constituents (% DM), a digestibility coefficient for each constituent (*D*) and potential biogas yield (*B*) of carbohydrates, XL and XP. *B* values have been estimated through stoichiometric equations and can be found in VDI 4630 (2004) as well as the average methane content of the biogas (*M*) produced by the different constituents. The CH<sub>4 sub</sub> is expressed in norm litres per kg volatile solids, which refers to the norm conditions of 273.2 K and 101.3 kPa:

$$\begin{array}{l} \mathrm{CH_{4\ sub}\ (l_{n}\ kg^{-1}\ VS) =\ (XF \times D_{(XF)} + NfE \times D_{(NFE)}) \times B_{(XF+NfE)} \times M_{(XF+NfE)} \\ + XL \times D_{(XL)} \times B_{(XL) \times} M_{(XL)} + XP \times D_{(XP)} \times B_{(XP)} \times M_{(XP)} \end{array}$$

In the present study D was calculated for each plot individually with respect to its dominant functional group, date of cut and the XF content of the total biomass. This was accomplished by linear regressions based on a dataset by University of Hohenheim (1997) (Table 1).

		8		(, * = = = )
FGD	constituent	Linear regression	$R^2$	RSE
Cross	XF	$D_{(\rm XE)} = -0.98 \times \rm{XF} + 102.34$	0.88	2.11
(700)	NfE	$D_{(NFF)}^{(AF)} = -1.27 \times XF + 107.82$	0.94	1.89
(> 70%  of biomass),	XL	$D_{(XL)}^{(E)} = 0.26 \times XF + 57.71$	0.80	0.78
1 <sup>st</sup> cut	XP	$D_{(XP)}^{(XL)} = -1.07 \times XF + 96.48$	0.95	1.48
T d bb	XF	$D_{(\rm XE)} = -1.75 \times \rm{XF} + 123.67$	0.95	2.03
Legumes and nerbs	NfE	$D_{(MF)}^{(MF)} = -2.42 \times XF + 136.16$	0.98	1.80
(> 30%  of biomass),	XL	$D_{\alpha I}^{(\text{NIE})} = -0.14 \times \text{XF} + 61.91$	0.01	6.47
1 <sup>st</sup> cut	XP	$D_{(XD)}^{(XD)} = -0.87 \times XF + 93.83$	0.91	1.33

Table 1. Linear regressions for grassland biomass from first cut to estimate digestibility values for Weende constituents according to functional group dominance (FGD) and XF content (% DM)

#### **Results and discussion**

The *D* values calculated by linear regression all yielded solid R2 values accept for XL. Here a R2 of only 0.01 occurred in the first cut while it was 0.59 in the second cut.  $CH_{4 sub}$  yields of 234 to 387  $l_n kg^{-1} VS$  and a mean of 299  $l_n kg^{-1} VS$  was observed across all plant species compositions and the two harvest dates. In the all functional group mixtures a mean  $CH_{4 sub}$  yield of 284  $l_n kg^{-1} VS$  was estimated for the first cut while the second cut was slightly higher with 296  $l_n kg^{-1} VS$  (Table 2). This is in line with results found by Gerstl (2008) from a two cut, extensively managed hill site in the Austrian Alps. Looking at the functional groups, the lowest  $CH_{4 sub}$  yields

were observed in the grass monocultures while legume monocultures where consistently high in both cuts (Table 2).

Table 2. Substrate specific and hectare specific methane yield of the different functional group monocultures and the all functional group mixtures

Eurotional aroun	Methane yield	Mathema wield (m <sup>3</sup> harl arl)	
Functional group	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	
Grasses*	270	289	1260
Legumes*	325	321	1317
s. Herbs*	336	300	865
t. Herbs*	304	317	892
all FG mixtures	284	296	1674



Figure 1. (A)  $CH_{4 \text{ sub}}$  yield and (B) annual  $CH_{4 \text{ area}}$  yield with increasing species richness. Error bars indicate standard error

The annual area-specific methane ( $CH_{4 area}$ ) yield, calculated as the product of annual biomass yield and  $CH_{4 sub}$  yield, was highest in the all functional group mixtures and similar to the values measured by Gerstl (2008). Between the functional group monocultures both herb fractions were about 300 m<sup>3</sup> ha<sup>-1</sup> a<sup>-1</sup> lower than legumes and grasses (Table 2). Along the species richness (SR) gradient a decline in  $CH_{4 sub}$  yield was observed with similar patterns in both cuts (Figure 1). This decline is due to an increase in less digestible XF with increasing SR. On the contrary, the annual  $CH_{4 area}$  yield doubled from the lowest (979 m<sup>3</sup> ha<sup>-1</sup> a<sup>-1</sup>) to the highest SR level (2027 m<sup>3</sup> ha<sup>-1</sup> a<sup>-1</sup>) due to a strong positive SR – biomass relation.

#### Conclusions

Calculating methane yields from Weende constituents and available digestibility data can give accurate estimates similar to those found in anaerobic digestion experiments with grassland biomass. Moreover, the method used here enabled us to account for a wide range in species composition and richness. Increasing species richness led to a decrease in  $CH_{4 \text{ sub}}$  yield whilst annual  $CH_{4 \text{ area}}$  yield increased two fold. Therefore, the overall benefit of species richness on anaerobic digestion characteristics of semi-natural grassland biomass is due to a strong species richness – biomass relation. These results highlight the potential of high diversity grasslands for energy production through anaerobic digestion.

#### References

Gerstl M. (2008) *Biogasbildungsvermögen von Grünlandbiomasse in Abhängigkeit vom Nährstoff- und Energiegehalt*, Diploma Thesis, University of Natural Resources and Applied Life Sciences, Vienna, Austria. Naumann C. and Bassler R. (2004) *Die chemische Untersuchung von Futtermitteln*, VDLUFA-Verlag, Darmstadt, Germany.

Roscher C., Schumacher J., Baade J., Wilcke W., Gleixner G., Weisser W.W., Schmid B. and Schulze E. (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology* 5, 107–121.

University of Hohenheim (1997) *DLG-Futterwerttabellen Wiederkäuer*, DLG-Verlag, Frankfurt, Germany. VDI 4630 (2004) *Vergärung organischer Stoffe*, Verein Deutscher Ingenieure, Düsseldorf, Germany.

# Evaluation of energy properties of *Phalaris arundinacea* hay compared with *Salix viminalis* wood

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## Abstract

The study assessed and compared the energetic properties of selected species of energy crops: *Phalaris arundinacea* hay and *Salix viminalis* wood. The basic properties of biomass power and heat: heat of combustion, calorific value, ash content, potassium, sodium, chlorine and sulphur, were determined in laboratory analysis. The analysis of energy properties of biomass revealed that biomass of *Salix vinimalis* is characterized by better energy properties with comparison to *Phalaris arundinacea* hay. The hay from *Phalaris arundinacea* grass was a less powerful fuel compared with *Salix vinimalis* because of its chemical composition and poor energy properties.

Keywords: biomass, energy properties, Phalaris arundinacea, Salix viminalis

## Introduction

The production of biomass in Poland is mainly based on *Salix* plantations (Piskier, 2008). However, experiments and small production areas also involve other energy plants. It is highly possible that the area where *Salix* and other energy plants are grown will increase because a decrease in the amount of arable areas can be observed. Also, there is a significant limitation in the use of green areas. Low quality meadows and pastures are no longer in use. EU subventions require that owners cut meadows and pastures, even those of the lowest quality, at least once a year. The outcome of such activity is hay that is not used due to low numbers of animals, or no animals, on farms.

The hay obtained from grass is considered to be a good quality fuel, and selected species of grass are classified as energy plants (Stolarski, 2008). Among the species of grass that can be used in the energy industry, there are both indigenous species and species that are not normally found in our environment (Bujak, 2008). Therefore, the energy characteristics of the hay obtained from an indigenous grass *Phalaris arundinacea* were compared with the wood of *Salix viminalis*, which is regarded to be a standard in energy industry.

## Materials and methods

The Łęczyńska Energetyka Ltd Company runs energy plant plantations on an area of 205.45 ha, divided into 190.75 ha of *Salix viminalis* and 14.7 ha of *Phalaris arundinacea*.

In November 2009, three years after the establishment of the plantation, samples of *Phalaris arundinacea* and *Salix viminalis* were taken from five randomly selected  $1 \text{ m}^2$  quadrats. Humidity of fresh mass and dry matter crop was measured. Dry hay and wood underwent specialist analysis, which aimed at assessment of usability of the obtained biomass for energy purposes.

Chemical composition of the biomass was determined by laboratory analysis. For this purpose, chemical mineralization of ground plant samples was conducted in mixture of nitric and perchloric acid, 3:1 ratio (Ostrowska *et al.*, 1991). Afterwards, the content of following elements: calcium, magnesium, potassium and sodium, was measured with AAS method.
Basic analysis of the energy properties of the biomass of the investigated plants were carried out in the chemical laboratory EC in Elblag. The results are the mean value of 5 measurements. Heat of combustion, lower heating value and ash content were measured, using standards methods described in Polish standards: [PN-81/G-04513] heat of combustion, lower heating value and [PN-80/G-04512] gravimetric method of ash measurement. In additional laboratory analyses, i.e. measuring content of carbon and sulphur using elemental-analysis method, and content of chlorine using Eschka method, were conducted, characterizing examined biomass as an energetic material. For the study, common analytical methods were used (Bątorek-Giesa and Jagustyn, 2009; Harkot *et al.*, 2007; Szyszlak-Bargłowicz and Piekarski, 2009).

The results were processed statistically. The significance of differences was tested using Tukey's test, with signifiance level of P = 0.05.

# **Results and discussion**

Independently of analysed species, result of high humidity of plant fresh mass was obtained (Table 1). This is considered an unfavourable feature with regard to energy value because humidity decreases effectiveness of combustion and causes a decline in the fuel value of biomass (Celińska, 2009). The mass of the investigated plants varied, being significantly higher for *Salix* in terms of dry matter crop.

Species	Humidity of fresh mass	Crop DM field
	(%)	(kg ha <sup>-1</sup> )
Salix viminalis	49.8	17 900
Phalaris arundinacea	50.6	14 100
LSD <sub>0.05</sub>	ns	3.584

Table 1. Humidity of fresh mass and dry matter crop of Salix viminalis and Phalaris arundinacea

ns – differences not significant.

The biomass of the investigated plants had high contents of alkaline compounds (Table 2). The presence of these compounds increases corrosion and accumulation of harmful deposits in the boiler during combustion of biomass. On the other hand, higher alkalinity of ash from biomass combustion causes a decrease in sulphur dioxide emission. This is due to the fact that calcium compounds found in ash act as an absorbent for sulphur oxides released in the process of combustion.

Table 2. Contents of alkanne elements in plant biomass (g kg * plant dry mass)										
Species	Ca	Mg	K	Na	Sum					
Salix viminalis	6.08	1.34	4.79	0.11	12.32					
Phalaris arundinacea	6.74	1.92	9.25	0.08	17.99					
Mean	6.41	1.63	7.02	0.09	15.16					
LSD <sub>0.05</sub>			5.254							

Table 2. Contents of alkaline elements in plant biomass (g kg<sup>-1</sup> plant dry mass)

The chemical analysis showed different contents of total sulphur and chlorine in the investigated grass and *Salix* (Table 3). *Phalaris arundinacea* had significantly higher contents of total sulphur and chlorine than *Salix viminalis*. The results of the study also show statistically significantly higher contents of ash from the biomass of *Phalaris arundinacea* (6.1%), as compared to *Salix viminalis* (1.7%). Similar results were obtained by Kalembasa (2006). The analysis showed low contents of ash and sulphur in the biomass, which is an important advantage over conventional coal (Celińska, 2009).

Specification	Salix viminalis	Phalaris arundinacea	LSD <sub>0.05</sub>
Total sulphur (%)	0.05	0.12	0.066
Chlorine (%)	0.02	0.26	0.223
Coal (%)	48.8	44.3	4.229
Flammable substance (%)	91.4	86.6	4.504
Ash (%)	1.7	6.1	4.085
Combustion heat (MJ kg <sup>-1</sup> DM)	18.63	17.00	1.557
Fuel value (MJ kg <sup>-1</sup> DM)	17.03	15.80	1.160

Table 3. Energy properties of biomass of Salix viminalis and Phalaris arundinacea

The analyses of energy properties of biomass obtained from different plants show that their heat value varies. Combustion heat of the investigated plant material, which is important from the point of view of energy industry, varied from 17.00 (*Phalaris arundinacea*) to 18.23 MJ kg<sup>-1</sup> DM (*Salix viminalis*). The combustion heat results for *Phalaris arundinacea* obtained in the experiment were lower than those found in Harkot *et al.* (2007), and similar to combustion heat values obtained by other authors (Dradrach *et al.*, 2007; Bujak, 2008). The combustion heat of *Salix* wood was similar to combustion heat of *Salix viminalis* had significantly higher values of combustion heat and fuel value than *Phalaris arundinacea*.

#### Conclusions

The biomass of energy plants *Salix viminalis* and *Phalaris arundinacea* differed in terms of chemical composition, crop yield, and energy content. The biomass of *Salix viminalis* had better energy properties than *Phalaris arundinacea*, smaller contents of ash, total sulphur, chlorine, and smaller sum of alkaline elements, with higher combustion heat and fuel value at the same time. The hay from *Phalaris arundinacea* turned out to be a poorer fuel than *Salix* because of its chemical composition and poor energy properties.

#### References

Bątorek-Giesa N. and Jagustyn B. (2009) Chlorine content in solid biomass used for power industry. *Ochrona Środowiska i Zasobów Naturalnych* 40, 396–401 (in Polish with English summary).

Bujak T. (2008) *Phalaris arundinacea*. How to manage wastewater from the production of yeast? *Agroenergetyka* 4(26), 11–13 (in Polish with English summary).

Celińska A. (2009) Characteristics of various energy crops in aspect of use in power industry. *Polityka Energetyczna*, 12, 2/1, 59–72 (in Polish with English summary).

Dradrach A., Gąbka D., Szlachta J. and Wolski K. (2007) Energy value of several grass species cultivated on light soil. *Grassland Science in Poland* 10, 29–35 (in Polish with English summary).

Harkot W., Warda M., Sawicki J., Lipińska H., Wyłupek T., Czarnecki Z. and Kulik M. (2007) The possibility of meadow sward use for energy purposes. *Grassland Science in Poland* 10, 59–67 (in Polish with English summary).

Kalembasa D. (2006) The amount and chemical composition of ash obtained from biomass of energy crops. *Acta Agrophysica* 7(4), 909–914 (in Polish with English summary).

Ostrowska A., Gawliński S. and Szczubiałka Z. (1991) Methods of analysis and evaluation of the properties of soils and plants. Instytut Ochrony Środowiska, Warszawa, 334 pp.

Piskier T. (2008) Energetic effectiveness of willow cultivation under various soil conditions. *Inżynieria Rolnicza* 2(100), 215–220 (in Polish with English summary).

PN-81/G-04513 - Solid fuels. Measurement of combustion heat and estimation of fuel value.

PN-80/G-04512 - Solid fuels. Measurement of ash content by gravimetric method.

Stolarski M. (2008) Energy crops plantations' condition. Czysta energia 6(80), 41-42.

Szyszlak-Bargłowicz J. and Piekarski W. (2009) Fuel value of biomass from virginia (*Sida Hermaphrodita Rusby*) stems depending on humidity. *Inżynieria rolnicza* 8 (117), 223–230 (in Polish with English summary).

# Grass for anaerobic digestion – methane production from five common grassland species at sequential stages of maturity

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# Abstract

This study investigated the effects of advancing plant maturity on the methane production of five grass species using small-scale, high-throughput batch digestion tests. Five common grass species (perennial ryegrass, Italian ryegrass, cocksfoot, timothy and tall fescue) were grown in field plots and harvested at three dates in the primary growth. Representative dry, milled herbage samples were used to determine methane production in 160 ml batch digestion tests. On average, total CH<sub>4</sub> production decreased numerically with advancing plant maturity, but this difference was not significant (P = 0.086). This trend of decreasing CH<sub>4</sub> production with advancing plant maturity did not differ (P > 0.05) across the five grass species investigated.

Keywords: anaerobic digestion, grass species, plant maturity, methane

## Introduction

Grassland represents the most significant biomass resource in Ireland, accounting for approximately 91% of the 4.3 million hectares of agricultural land. Consequently, grass will be a dominant feedstock for anaerobic digestion on Irish farms. Grass can be an excellent energy crop and may be classified as a high yielding (up to 20 t DM ha<sup>-1</sup> a<sup>-1</sup>), low input perennial crop. Two of the main grassland management factors affecting herbage chemical composition are grass species and plant maturity at harvest. Grass species differ in productivity and chemical composition and this may impact on their methane production potential. Advancing maturity of grass from the vegetative to the inflorescence growth stage is characterised by an increase in fibre components and a decrease in digestibility (Stefanon *et al.*, 1996). Herbage quality is generally considered to decline with advancing plant maturity, so delaying the harvest date can have a negative influence on methane production (Prochnow *et al.*, 2009). This study investigated the effects of advancing plant maturity of five grass species on methane production using dried, milled samples in a small-scale (160 ml), high-throughput batch digestion test.

#### Materials and methods

Five common grass species: perennial ryegrass (PRG; *Lolium perenne* L. cv. Gandalf), Italian ryegrass (IRG; *Lolium multiflorum* Lam. cv. Prospect), cocksfoot (*Dactylis glomerata* L. cv. Pizza), timothy (*Phleum pratense* L. cv. Erecta) and tall fescue (*Festuca arundinacea* Schreb cv. Fuego), were grown in field plots (within three replicate blocks) under an inorganic nitrogen fertiliser input of 125 kg N ha<sup>-1</sup> and separate plots were harvested at three dates (12 May, 9 June and 7 July; Harvests 1 to 3; n = 45) in the primary growth. On each harvest date, total solids (TS) concentration was estimated following drying at 98°C for 16 h. Replicate samples were also dried at 40°C for 48 h before being milled. Dried, milled samples were used for the determination of *in vitro* dry matter digestibility (DMD), neutral detergent fibre (NDF) and ash (volatile solids (VS) = TS – ash) as previously described by Purcell *et al.* (2011). Replicate milled samples were also analysed for methane production in 160 ml batch digestion tests, according to VDI guideline 4630 (2006). Briefly, substrate and inoculum were

added to the bottles at a volatile solids (VS) inoculum to substrate ratio of 2:1 and at a final VS concentration of 10 g kg<sup>-1</sup>. The inoculum was sourced from a cattle slurry digester at the Agri-Food and Biosciences Institute in Hillsborough, Northern Ireland. Micro- and macro- mineral solutions were added to ensure that nutrient conditions in the bottles were not limiting and sodium hydrogen carbonate was added as a buffer system (3.5 g L<sup>-1</sup>). Water was added to each bottle to adjust the final volume to 70 ml, the pH was adjusted to 7.2 and the bottles were flushed with N<sub>2</sub> and sealed with butyl rubber stoppers. Six replicate bottles with inoculum only (blanks) were also included. Bottles were incubated at 38°C and mixed daily. The gas headspace pressure inside each bottle was recorded after 2, 5, 8, 13, 18, 27 and 35 days incubation using a detachable pressure transducer and the total amount of gas produced was estimated. A 0.8 ml sample of this gas was used to determine CH<sub>4</sub> concentration by gas chromatography (Purcell *et al.*, 2011).

Data were analysed as a split-plot design using the Proc MIXED procedure of SAS, Version 9.1.2 (SAS, 2004) with harvest date as the main plot and grass species as the sub-plot, and accounting for repeated measures effect of sampling day (batch digestion data only).

#### **Results and discussion**

Of the five grass species investigated in this study, the NDF concentration was lowest (P < 0.001) for the IRG followed by the PRG, while cocksfoot had the lowest (P < 0.05) DMD (Table 1).

Grass species	Harvest	TS	VS	DMD	NDF (g kg <sup>-1</sup> DM)	Total $CH_4$ production
	1	168	905	785	496	275
Perennial ryegrass	2	220	923	702	609	263
	3	233	928	569	635	250
	1	194	910	748	501	268
Italian ryegrass	2	231	920	708	543	252
	3	214	914	635	587	251
	1	166	907	804	552	268
Timothy	2	191	918	710	654	272
	3	221	931	598	659	245
	1	180	904	738	518	261
Cocksfoot	2	221	908	653	622	249
	3	229	906	531	686	251
	1	184	914	710	529	258
Tall fescue	2	213	910	680	623	235
	3	217	916	582	653	230
Standard error of the	mean					
Species		3.7	2.6	15.6	4.9	6.0
Harvest		4.7	2.1	15.2	5.5	4.7
Species × Harvest		8.2	3.7	26.3	9.5	10.4
Levels of significance	e					
Species		**	ns	**	***	ns
Harvest		ns	***	*	***	ns
Species × Harvest		ns	**	ns	***	ns

Table 1. Effect of grass species and advancing plant maturity on grass chemical composition (g kg<sup>-1</sup>, unless otherwise stated) and total  $CH_4$  production (L  $CH_4$  kg<sup>-1</sup> VS added)

On average for the 35-day batch digestion tests, 0.64 of the total  $CH_4$  was produced by day 8 of the test. The specific methane yield varied from 230 (cocksfoot, Harvest 3) to 275 L  $CH_4$  kg<sup>-1</sup> VS<sub>added</sub> (PRG, Harvest 1). Of the five grass species investigated, average

daily  $CH_4$  production was highest (P < 0.05) for the PRG, while the apparent rate of digestion was lowest (P < 0.001) for the cocksfoot, with other grasses not differing (data not shown). In temperate grassland regions, PRG is preferred for animal production because of its high digestibility and water soluble carbohydrate content, and reduced fibre concentration. These factors combined in the current study to produce the highest methane yield. On average, herbage NDF concentration increased (P < 0.001) with advancing plant maturity (519, 610 and 640 g kg<sup>-1</sup> DM for Harvests 1, 2 and 3, respectively), reflecting the general decrease in plant leaf to stem ratio and the increasing cell wall content as the plant matured (Stefanon *et al.*, 1996). This was accompanied by a decrease (P < 0.01) in herbage DMD. This resulted in an apparent slower rate (P < 0.001) of digestion in batch tests with advancing harvest date (Table 1 and Figure 1). Although total  $CH_4$  production decreased numerically with advancing plant maturity, this difference was not significant (P > 0.05). This trend of decreasing  $CH_4$  production with advancing plant maturity did not differ (P > 0.05) across the five grass species investigated.



Figure 1. Effect of advancing plant maturity on cumulative  $CH_4$  production in 35-day batch digestion tests (averaged across grass species; SEM = 1.2; P < 0.001)

#### Conclusions

Of the five grass species investigated in this study, PRG may be most desirable as a feedstock for biogas production. Although the total  $CH_4$  produced decreased numerically with advancing plant maturity, this trend did not reach statistical significance. Future work will use regression analysis to further compare these grass species at similar phenological growth stages.

#### References

Prochnow A., Heiermann M., Plochl M., Linke B., Idler C., Amon T. and Hobbs P.J. (2009) Bioenergy from permanent grassland – a review: 1. Biogas. *Bioresource Technology* 100, 4931–4944.

Purcell P.J., O'Brien M., Boland T.M. and O'Kiely P. (2011) In vitro rumen methane output of perennial ryegrass samples prepared by freeze drying or thermal drying (40°C). *Animal Feed Science Technology* 166–167, 175–182.

SAS (2004) SAS for windows (Version 9.1.2). Statistical Analysis System Institute Inc., Cary, North Carolina, USA.

Stefanon B., Pell A.N. and Schofield P. (1996) Effect of maturity on digestion kinetics of water-soluble and water-insoluble fractions of alfalfa and brome hay. *Journal of Animal Science* 74, 1104–1115.

VDI 4630 (2006) Fermentation of organic materials – Characterisation of the substrate, sampling, collection of material data, fermentation tests. The Association of German Engineers, Düsseldorf, Germany.

# The biogas potential of Juncus effusus L. using solid phase fermentation technique

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# Abstract

Wet grassland sites are often infested with *Juncus effusus* L. (soft rush). A high coverage of soft rush will lead to a restricted use in traditional animal-based farming systems. Therefore alternative options of biomass use are required urgently. Biogas production using solid-phase fermenters could be a promising alternative for the use of soft rush-infested swards. In our experiment, we varied the amount of soft rush (100% *Juncus*; 50% *Juncus* + 50% grasses and herbs; 100% grasses and herbs) in the substrate and analysed the substrate compaction. We then determined the methane production and a range of chemical and physical substrate parameters before and after anaerobic digestion. Methane yields of *Juncus* (100%) were smaller than that of the accompanying grassland vegetation. The methane yield of the grass-Juncus-mixture was higher than expected from the energy content. We found that Juncus contributed to substrate stability and presumably to gaseous diffusion of methane through the substrate stock.

Keywords: soft rush, biomass utilization, biogas, methane

# Introduction

Wet grassland sites are often infested with soft rush (*Juncus effusus* L.) due to wetland restoration measures (Nielsen and Hald, 2010). To sustain typical wet grassland vegetations and to avoid further succession towards reeds and shrubs, a regular utilisation is necessary. However, a high coverage of soft rush will lead to a limited biomass quality (Odeyinka *et al.*, 2006), with a restricted use in traditional animal-based farming systems (Cherrill, 1995). To maintain wet grasslands, alternative options of biomass use are required urgently. Biomass use via methanogenesis for energetic purposes is often regarded as an option. Wet fermentation plants are widespread, but not well suited to substrates rich in lignocelluloses. The solid phase fermentation technique (SPFT) is better adapted to high-fibre substrates than common wet fermentation techniques. Therefore the aim of our investigations was to study the capability of soft rush as a substrate for SPFT.

# Materials and methods

The experiments on the methanogenesis potential of soft rush, that is the substrate performance in an SPFT percolation process, were conducted in 10 laboratory-scale batch fermenters (120 l). The experimental set-up is given in Figure 1. We tested five substrates which originated from peat grassland swards with high coverage of *Juncus effusus* (Table 1). Biomass was collected from an autumn growth, chopped by hand to an average length of 5 cm and ensilaged in plastic tubs. Material from each substrate was filled in two 120 l fermenters. The methanogenesis was started by sprinkling process water over the stacked biomass. The temperature in the fermenter was controlled and kept in the mesophilic range over the 60 d measuring period.

Each fermenter was percolated three times per day. The total volume of biogas produced was measured daily by a drum-type gas meter.



Figure 1. Experimental setup - schematic view of a single 120 l fermenter

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Location	ID	Grasses (%)	Herbs (%)	Soft rush (%)	Classification
Emsland	Ι	1	1	98	Juncus
Emsland	II	40	10	50	Mix
Darß	III	100	0	0	Grass
Darß	IV	50	0	50	Mix
Darß	V	0	0	100	Juncus

Table 1. Substrate classification and composition (percentage of dry matter)

The biogas composition was analysed by infrared detection (Bernt DGA 3) for  $CH_4$ ,  $CO_2$ , and  $O_2$ . Biogas and methane yields were converted to standard conditions (T = 273.15 K; P = 101.325 kPa).

#### Results

Methane yields of *Juncus* were smaller than that of the accompanying grassland vegetation (Figure 2). Methane yield of the mixed substrate did not differ significantly from that of either Grass extensive or *Juncus*, but was higher than expected from estimations based on feed analysis according to Kaiser (2007).

The substrate compaction had an effect on feedstock specific biogas yield (Figure 3). With increasing substrate compaction the biogas yield per unit of oDM of the *Juncus* substrates declined (Figure 3a) while that of the mixed substrate increased (Figure 3b).

The *Juncus* substrate yielded lowest in methane, but production of biogas continued to the end of the 60 d measuring period.



Figure 2. Substrate specific methane production in a solid phase fermentation process (retention time 60 days, error bars are the standard deviations and different letters indicate significant differences of the means (P < 0.05; T-test), \* = estimated methane yields according to Kaiser,  $l_N kg^{-1} oDM^{-1} = norm litre per kg organic dry mass, reference substrates "Grass intensive" and "Maize" were frequently percolated)$ 



Figure 3. The relationship between substrate compaction and biogas yield in SPFT (with trend line, g DM  $l^{-1}$  = g of substrate dry mass per litre;  $l_N g^{-1} \text{ oDM}^{-1}$  = norm litre per g organic dry mass)

#### **Discussion and conclusions**

The feedstock specific methane yields of silages from pure stands of Juncus were smaller than those from pure grass stands, but still within a range of common late-cut biomass from extensive grasslands (Prochnow et al., 2009). Juncus substrate in this experiment was cut at a relatively late physiological stage. If young Juncus would be used and would than be preconditioned and percolated more frequently there seems to be a realistic potential of improved methane yields from Juncus dominated grassland swards. Moreover, there seems to be a positive additional effect of Juncus as a component in a mixture with grass dominated substrates. Ashekuzzaman and Poulsen (2010) have also shown that using multi-component substrates increases the methane yield by more than would be expected from the digestion of single substrates. We assume that in our experiments this was due to the specific physical-structural properties of soft rush. A greater stability in the stack accompanies a higher percolate absorption ability. The gas exchange in the mixed-treatment stacks might have been improved by the presence of tubular aerenchyma from the soft rush particles. We conclude that biogas production using a solid-phase fermenter can be a promising alternative for the use of soft rush infested swards once substrate conditioning as well as the fermentation process management have been optimised.

#### References

Ashekuzzaman S.M. and Poulsen T.G. (2010) Optimizing feed composition for improved methane yield during anaerobic digestion of cow manure based waste mixtures. *Bioresource Technology* 102(3), 2213–2218. Cherrill A. (1995) Infestation of improved grasslands by *Juncus effusus* L. in the catchment of the River Tyne, Northern England: a field survey. *Grass and Forage Science* 50, 85–91.

Kaiser F. (2007) Einfluss der stofflichen Zusammensetzung auf die Verdaulichkeit nachwachsender Rohstoffe beim anaeroben Abbau in Biogasreaktoren. PhD thesis. Technical University of Munich, 176 pp.

Nielsen A.L. and Hald A.B. (2010) Shortcut strategies to improve plant species richness after years of intensive management in moist grassland. *Grassland Science in Europe* 15, 1052–1054.

Odeyinka S.M., Hector B.L. and Ørskov E.R. (2006) Nutritive evaluation of some trees and browse species from Scotland. *European Journal of Scientific Research* 14(3), 311–318.

Prochnow A., Heiermann M., Plöchl M., Linke B., Idler C., Amon T. and Hobbs P.J. (2009) Bioenergy from permanent grassland – a review: 1. Biogas. *Bioresource Technology* 100(21), 4931–4944.

# Factors affecting the carbon content in reed canary-grass (*Phalaris arundinacea* L.) used for producing burning material in Latvia

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# Abstract

Reed canary grass is used for fuel briquettes and pellet production. The objective of this research was to evaluate the factors influencing the carbon content of reed canary grass (*Phalaris arundinacea* L.). The field trials were conducted using the cultivars Marathon and Bamse sown in a sod–podzolic loam soil in August 2008 (Marathon) and April 2009 (both cultivars). The carbon content of harvested samples averaged 0.37 g kg<sup>-1</sup> dry matter. Generally, the older the plant, the greater was the carbon content. Of all the factors investigated (cultivar, nitrogen fertilizer rates and sowing time), the cultivar had the greatest impact on the yield and chemical content of reed canary grass. The sowing time and nitrogen fertilizer rate influenced the yield, as well as the ash and carbon content.

Keywords: Phalaris arundinacea, carbon content, N fertilizer, fuel production

# Introduction

Carbon is the quantitatively most important element in living organisms. Therefore, when organic material is burnt, gaseous carbon compounds, especially carbon dioxide ( $CO_2$ ), also form an important part of the products. In the atmosphere, carbon is found in carbon monoxide (CO),  $CO_2$  and methane ( $CH_4$ ); gases fundamentally affecting global warming and consequently the climate on earth (Klaviņš *et al.*, 2008). The use of biomass for energy production is increasing worldwide and is supposed to provide clean and sustainable energy. In several countries, reed canary grass (*Phalaris arundinacea* L.), a perennial C3 species, is gaining importance for the production of fuel briquettes and pellets. In this study, we wanted to evaluate factors influencing the carbon content in the biomass, as well as the yield, of *P. arundinacea*, and thus assess the sustainability of its use as a bioenergy plant.

# Materials and methods

Three field experiments with reed canary grass cultivar Marathon were carried out in a sodpodzolic loamy soil (organic matter content of the soil was 5.2%, pH <sub>KCl</sub> was 5.8, P<sub>2</sub>O<sub>5</sub> was 20 mg kg<sup>-1</sup>, and K<sub>2</sub>O was 90 mg kg<sup>-1</sup> of soil). The area per plot was 16 m<sup>2</sup> and the location of the plots was randomised. The reed canary grass was sown after a fallow period. Reed canary grass cultivar Marathon was sown on 12 August 2008 (henceforth Marathon 08) and both cvs Marathon and Bamse again on 29 April 2009 (henceforth Marathon 09 and Bamse 09). Before sowing, 400 kg ha<sup>-1</sup> of NPK-fertilizer was applied (N:P:K-5:10:25). Supplementary N fertilizer was applied to cvs Marathon 08 on 20 May 2009, and to Marathon 09 and Bamse 09 on 22 July 2009 at the following rates: 0, 30, 60 or 90 kg N ha<sup>-1</sup>, respectively (labelled N0 to N90). The dry matter production was sampled from 0.25 m<sup>2</sup> areas on three replicates on 12 October 2009, 6 April 2010 and 6 October 2010.

Carbon content in samples was determined using the standard ISO 625. Concentrations of arsenic (As), cadmium (Cd), lead (Pb) and other elements in the reed canary grass samples were measured with an inductively coupled plasma optical emission spectrometer (Perkin Elmer Optima 2100 DV). The ash content was quantified with the accelerated standard method, and lignin content with Clason's methods. In 2009, there was a drought, with a third of the usual rainfall in May, despite air temperatures close to the long-term average. The trial data were processed using correlation, regression and variance analyses (ANOVA) and descriptive statistics with Excel for Windows 2000 (Arhipova and Bāliņa, 2006). The means are presented with their LSD test.

## **Results and discussion**

The mean carbon content in the reed canary grass samples was  $0.383\pm0.05$  g kg<sup>-1</sup> (Figure 1). It was influenced by the sampling period and the age of the plants; in the first year the autumn crop was within the range of 0.350-0.406 g kg<sup>-1</sup> and in the second year between 0.366-0.418 g kg<sup>-1</sup>. The largest carbon content was found in the reed canary grass cv. Marathon 09. The largest mean carbon content was achieved in treatments with 90 kg N ha<sup>-1</sup>. Bridgeman *et al.* (2008) reported carbon contents of reed canary grass of 0.486 g kg<sup>-1</sup>, which was nearly 10% more than our values.

Carbon yield ranged between 1.09 and 3.89 t ha<sup>-1</sup> (Figure 1). It was dependant on the cultivar, plant age and the N fertilizer application. The reed canary grass cv. Marathon 08



Figure 1. The carbon (C) content and the carbon yield for reed canary grass dependant on the sowing period, the cultivar and the nitrogen (N) supplementary fertilizer norms (kg N  $ha^{-1}$ )

first-year samples have a greater carbon yield than the autumn samples of cv. Marathon 09. Different N fertilizer norm is a fundamental factor which influences the yield (Wrobel *et al.*, 2009).

A significant (P < 0.05) linear correlation between the carbon content and cadmium, silicon and sodium content was found in the reed canary grass samples (P < 0.05; n = 36). Wrobel *et al.* (2009) have shown that a high silicon concentration influences the burning properties of reed canary grass (Figure 2).



Figure 2. The connection between carbon content and ash, and chemicals elements (P < 0.05) in reed canary grass cultivars

#### Conclusions

The carbon content in reed canary grass samples was on average  $0.383\pm0.05$  g kg<sup>-1</sup>. If we evaluate the influence of N application on quantity changes, the largest mean carbon content occurred when using 90 kg N ha<sup>-1</sup>. There were significant (P < 0.05) linear correlations between carbon content and cadmium, silicon and sodium content. The carbon yield per hectare was within the range 1.09–3.89 t ha<sup>-1</sup>, depending on the cultivar, plant age, and the N fertilizer amount.

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#### References

Arhipova I. and Bāliņa S. (2006) Statistika ekonomikā (Statistics in economy). Datorzinību centrs, Rīga, 352 pp.

Bridgeman T.G., Jones J.M., Shield I. and Williams P.T. (2008) Torrefaction of reed canary grass, wheat straw and willow to enhance solid fuel qualities and combustion properties. *Fuel* 87, 844–856.

Cars A. (2008) Energoresursi. Rīga: SIA Baltic Communication Partners, 102 pp.

Kļaviņš M., Nikodemus O., Segliņš V. et al. (2008) Vides zinātne (Environmental Science). Rīga. Latvijas Universitāte. 599 pp.

Wrobel C., Coulman B.E. and Smith D.L. (2009) The potential use of reed canary grass (*Phalaris arundinacea* L.) as a biofuel crop. *Acta Agriculturae Scandinavica* 59, 1–18.

# Optimisation of sustainable biomass supply from representative permanent grassland for thermal use

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## Abstract

In a cooperative project GNUT (2008–2011) the focus was on the availability and suitability of permanent grassland with a maximum of two cuts per year, its cultivation requirements, and applicability for combustion. The project GNUT will provide urgently needed knowledge to make the energetic potential of permanent grassland accessible. Additionally, specific conditions of use, like conservation restrictions, are considered. The strategies will be evaluated by ecological and economic criteria. For the study, five permanent grassland types typical for Germany have been evaluated. The grassland types have been categorized by different natural conditions and tested under various cultivation intensities (cutting frequency, time of cutting and fertilisation). The biomass yield and biomass quality, contents of parameters that are important for combustion, and ash melting points were measured. The biomass potential of the grassland types ranged from 2.3 Mg DM ha<sup>-1</sup> to 15.5 Mg DM ha<sup>-1</sup>. The grassland types with the highest annual biomass yields always had the highest contents of inorganic substances, especially micro nutrients and heavy metals. These are responsible for aerosol emissions, corrosion processes and slag formation.

Keywords: permanent grassland, thermal use, combustion, biomass quality

# Introduction

Permanent grassland has a multitude of functions. The realisation of these functions is dependent upon how the land is managed (Elsäßer, 2006). Fodder originating from extensively cultivated permanent grasslands is rather unsuitable for use as dairy cattle feed (Hochberg and Zopf, 2010). Furthermore, as dairy farming uses more feedstock from arable land, the traditionally grazed permanent grasslands are left unused. This necessitates that new uses are found for the grasslands; uses that acknowledge the importance of nature conservation and ensure the preservation of native plant communities. One such use could be the production of hay derived from this land for use as a solid fuel.

# Materials and methods

Table 1 details the permanent plant communities at 5 different sites, and the four management variants that were applied at each site. These variants differ in the time of cutting, cutting frequency, and the degree of fertilisation. To be able to evaluate changes in botanical composition during the experiment the number of species, the grassland's value and the diversity of the plant community were estimated. The dry matter (DM) yield was measured from the biomass, and the chemical composition was examined, for instance N, K, Cl and S as elements responsible for corrosion and emission (VDLUFA Vol. III). Additionally, ash melting reaction (DIN CEN/ TS 15370–1) were also determined for selected cuts.

Tuno	Variant	Cut frequency	and time of cut	Fertilisation kg ha <sup>-1</sup>			
Туре	variant	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	Ν	Р	Κ	
	1	2. July	30. September				
Carriesterre restrictes	2	9. August					
Caricelum rostralae	3	30. September					
	4	biannual at	t 18. August				
	1	13. June	27. September				
	2	13. June	27. September			140	
Phalariaetum arunainacea	3	27. September					
	4	biannual at 2	28. September				
	1	3. July	1. October				
Angelico sylvestris-Cirsietum	2	1. August	24. October				
oleracei	3	17. August					
	4	biannual at	t 19. August				
	1	22. June	7. September	60	20	150	
Geranio sylvatici-Trisetum	2	22. June	7. September		20	110	
flavescentis	3	22. June	7. September				
	4	biannual at 1	7. September				
	1	18. August					
Molinian agamulaga	2	13. September					
mounion caerulede	3	17. July	13. September				
	4	biannual at 1	7. September				

Table 1. Cutting frequency and fertilisation of the four management variants at the tested grassland types

#### Results

The main aim, to ensure the preservation of the plant community with a minimum of cultivation, was achieved with a variety of variants for the different plant communities. For *C. rostratae* Variant 1 led to a maximum annual yield of 9.0 Mg DM ha<sup>-1</sup>, whilst a biannual yield of 6.2 Mg ha<sup>-1</sup> was obtained by Variant 4. The *P. arundinaceae* gave a maximum yield of 15.5 Mg DM ha<sup>-1</sup> by Variant 2, and produced 9.8 Mg DM ha<sup>-1</sup> annually by Variant 3. Variant 3 and Variant 4 lead to annual DM yields of between 4.4 Mg ha<sup>-1</sup> and 4.6 Mg ha<sup>-1</sup>, and are suitable strategies for the preservation of the *A. sylvestris-C. oleracei*. The *G. sylvatici-T. flavescentis* community produced a yield of 5.7 Mg DM ha<sup>-1</sup> when Variant 2 was applied and a yield of 3.4 Mg DM ha<sup>-1</sup> under Variant 3. In the case of *M. caeruleae* the grassland is best preserved by Variant 2, which produced a yield of 2.3 Mg DM ha<sup>-1</sup>. Here on, only the management variants that best preserve the grassland are considered.

DM and the ash melting temperature in °C by Variants that preserve the plant community									
	Variant	Ν	Cl	K	S	ash melting point			
	1-1	15.6	6.4	12.9	2.2				
C. rostratae	1-2	17.2	6.0	11.7	2.3	853-913			
	4	13.1	3.3	6.1	1.6				
	2-1	12.6	11.0	17.9	2.4				
P. arundinaceae	2-2	17.4	9.3	10.3	2.9	903-1070			
	3	16.3	8.0	8.6	2.7				
A subsection C share si	3	20.0	9.1	16.7	2.3	1015			
A. sylvestris-C. oleracel	4	16.0	5.6	9.4	1.7	1015			
	2-1	15.7	10.7	19.5	1.3				
C militatioi T flancas antis	2-2	23.3	10.3	19.4	2.0	1170 1260			
G. sylvalici-1. flavescentis	3-1	13.9	4.8	10.7	1.9	11/0-1500			
	3–2	18.9	2.4	8.0	3.2				
M. caeruleae	2	13.8	1.9	9.6	1.2	1120			

Table 2. Concentrations of the elements responsible for corrosion and emission: N, Cl, K, S in g kg<sup>-1</sup> DM and the ash melting temperature in  $^{\circ}$ C by Variants that preserve the plant community

The concentrations of the elements responsible for corrosion and emission (N, Cl, K, S) and the ash melting point, which are found in the different grasses harvested using the different strategies, are shown in Table 2. The highest concentrations of these elements were found in those grasses treated with fertilisers, and in *A. sysvestris- C. oleracei* produced by Variant 3. The ash melting points of *M. caeruleae* and *G. sylvatici-T. flavescentis* were highest. Somewhat lower were *C. rostratae*, *P. arundinaceae* and *A. sylvestris-C. oleracei*. The lowest ash melting temperature, and thus the most unfavourable for use as solid fuel, was given by *C. rostratae*.

#### Discussion

The fundamental suitability of the different grassland growths for use in combustion have been compared with straw (Rösch *et al.*, 2007; Hering *et al.*, 2008), and the former have been found to contain higher concentrations of the polluting elements. The use of additional fertilisation led to grasses containing higher concentrations of K and Cl. A later harvest or a biannual harvest can both lead to favourably lower concentrations of these elements. Except for *C. rostratae*, the ash melting points of the grasses were higher than that of straw, so this should not lead to increased slag formation compared with conventional fuels.

#### Conclusions

Permanent grasslands are important in terms of nature conservation. Moreover, the biomass produced from these grasslands contains favourably higher concentrations of the ingredients disadvantageous for combustion. Using such grasses places high demands upon the combustion technologies, but these challenges are not insurmountable. Through the application of minimally intensive plant cultivation measures it is possible to partially optimise grasses for use in combustion.

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#### References

Elsäßer M. (2006) Nichtproduktionsfunktionen von Dauergrünland: Möglichkeiten und Grenzen der Ökologisierung der Landwirtschaft. In: Biologische Bundesanstalt für Land- und Forstwirtschaft Berlin und Braunschweig Möglichkeiten und Grenzen der Ökologisierung der Landwirtschaft – wissenschaftliche Grundlagen und praktische Erfahrungen – Beiträge aus dem Arbeitskreis Agrarökologie Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem.

Hering T., Scheftelowitz M. and Peisker D. (2008) Abschlussbericht des Teilprojektes Bereitstellung und Analytik von Referenzbrennstoffen im Projekt Feinstaubemissionen aus Kleinfeuerungsanlagen für Getreide- und Strohbrennstoffe des IE gGmbH, Leipzig. Thüringer Landesanstalt für Landwirtschaft (TLL), Jena. Hochberg H. and Zopf D. (2010) Möglichkeiten der Nutzung und Verwertung von Extensivgrünland In: Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA) *Landschaftselement oder Rohstofflieferant – zur multifuktionalität des Grünlandes.* 122. VDLUFA-Kongress, VDLUFA-Verlag, Speyer, pp. 107–116.

Rösch C., Raab K., Skarka J. and Stelzer V. (2007) Energie aus dem Grünland – eine nachhaltige Entwicklung? Forschungszentrum Karlsruhe in der Helmholtz-Gemeinschaft. FZKA 7333.

# Biomass potential of *Miscanthus x giganteus* grown in Lithuania for bioenergy purposes

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# Abstract

Field trials were conducted in Dotnuva, Lithuania (55°24' N, 23°52' E) on *Miscanthus x* giganteus cultivation for bioenergy. The plant was tested for tolerance of climate and environmental conditions: over winter survival, growth rate and development characteristics in the first four cultivation years, biomass potential, and the effect of nitrogen (N) fertilisers on the productivity. *Miscanthus* seedlings (2-leaf stage) were introduced from Austria and were planted at a rate of 2 seedlings per m<sup>2</sup>. Biomass yield and its chemical composition were determined at the end of each growing season. The *Miscanthus* persistence after 4 years of cultivation was 54%. *Miscanthus* biomass yield increased from on average 6 t ha<sup>-1</sup> dry matter (DM) in the second year to 11 t ha<sup>-1</sup> (DM) in the third year. Due to the cold and snowy winter of 2009–2010, the 3-year-old *Miscanthus* stand severely thinned out and the yield declined in the fourth year of growth to 7.2–7.8 t ha<sup>-1</sup>. The concentrations of N, carbon (C) and sulphur (S) in *Miscanthus* biomass was more dependent on the stand age rather than on the level fertilisation. The hybrid *Miscanthus* can be recommended for growing in Lithuania for bioenergy purposes.

Keywords: bioenergy, biomass, fertilization, Miscanthus x giganteus, productivity

# Introduction

In the countries with a cooler climate, the spread of *Miscanthus* species is constrained by its susceptibility to contrasting temperatures, especially to low temperatures, and by the fact that the plants do not produce ripened seed (Lewandowski *et al.*, 2000). The distinct advantages of *Miscanthus* x *giganteus* hybrid are its long productive age, and biomass use as a renewable energy source and for lignin cellulose production (Heaton *et al.*, 2008). The biomass of *Miscanthus* (a  $C_4$  type plant) was found to contain much more carbohydrates compared with legumes and  $C_3$  type plants, and this facilitates its use for energy production (Pappas *et al.*, 2009). An especially high emphasis is placed on the effect of *Miscanthus* on the environment, which is very favourable and beneficial for the maintenance of sustainable ecosystems. Given that global climate-change processes and climate warming are beneficial for the growth of  $C_4$  type plants, the aim of this study was to determine the adaptation of *Miscanthus* x *giganteus* to Lithuania's climate and environmental conditions.

# Materials and methods

The trials were conducted at the Institute of Agriculture in Dotnuva ( $55^{\circ}24'$  N,  $23^{\circ}52'$  E), in a reclaimed river-bed territory. The soil is light sand on sand with small stone and gravel admixture (*Eutri-Cambic Arenosols*). The soil is neutral, alkaline at depth, with a humus status of 2%, total nitrogen is moderate at 0.14%, and content of available phosphorus is 122 mg kg<sup>-1</sup> and potassium 166 mg kg<sup>-1</sup>. The pre-crop plant was red clover in its third year of use. In July 2007, seedlings of the *Miscanthus* hybrid grown to the 2–4 leaf stage were obtained from Austria. The seedlings were planted in 10 m-long rows, laid out in three

replications, with row spacings of 1 m and 0.5 m intervals between plants in the rows (2 plants m<sup>-2</sup> or 20 000 plants ha<sup>-1</sup>). *Miscanthus* was not cut in the autumn of the first year of cultivation, and the stands were mulched with winter wheat straw. The plants were assessed for the following parameters: establishment, growth, tillering or emergence of lateral shoots, and overwinter survival. In the first year, after the plants had become established, compound fertilisers (16% N, 16% phosphorus (P) and 16% potassium (K)) were applied at a rate 400 kg ha<sup>-1</sup>. Three nitrogen (N) fertilisation levels (0, 60 and 120 kg ha<sup>-1</sup>) were investigated from the second year of growing. The experiment was designed as a randomized complete block with three replicates. To estimate biomass yield, the plants were cut at the end of the vegetation period and fresh mass was immediately weighed. Chemical analyses were carried out on composite chopped mass samples taken from 3 replications, then dried and ground. The results were processed using ANOVA.

#### **Results and discussion**

Ten days after Miscanthus planting, 88% of the 348 seedlings planted had established. The established plants grew rapidly and developed intensively until the autumn frosts. After the mild winter of 2007–2008 and an early spring with contrasting temperatures, vegetative growth started late, in the middle of May. Part of the shoots that had started to emerge were susequently frost-killed at the end of May. At the beginning of intensive growth (June) 219 plants were recorded. Straw mulch remained undecomposed during the whole vegetation period of the second year. After the second winter of 2008-2009, in spring, Miscanthus started vegetation earlier than after the first winter. All plants survived the winter and started vegetative growth. Due to the cold and snowy winter of 2009-2010, the 3-year-old stand of *Miscanthus gigantus* became severely thinned out. In the spring of 2010, in the fourth year of growth, the plants that survived accounted for 54% of the total number that had been planted in 2007. In the above-ground biomass of the second-year *Miscanthus* that was cut upon completion of season's vegetation, leaves dominated and accounted for on average 55% of biomass However, in the third year of growth, the stems grew very tall and the leaves of the bottom part started drying early; therefore, their share in the total dry biomass in the autumn dramatically declined and made up on average only 29%. Some literature findings also indicate that with increasing productive age of *Miscanthus* stands, the share of stems in the total biomass can increase up to 92% and this has a direct effect on biomass energy value (Christian et al., 2008). Nitrogen fertiliser effect on biomass structure was noted only in the second-year stands. The higher N-fertiliser rate  $(N_{120})$  tended to increase plant leafiness compared with the N60 rate. Fresh biomass yield of the second-year plants that grew during the dry growing season of 2008 varied from 15.8 to 24.7 t ha-1, and dry biomass varied from 4.6 to 6.8 t ha<sup>-1</sup> (Table 1).

Table 1	. The	impact	of nitrogen	fertiliser	on	Miscanthus	biomass	dry	matter	yield	in t	he	autumn	of
the seco	ond, th	nird and	I fourth year	r of grow	th (	t ha <sup>-1</sup> )								

Nitrogen fertilisation -		Auorogo		
	Second (2008)	Third (2009)	Fourth (2010)	Average
N <sub>0</sub>	4.62	10.50	4.88	6.67
N <sub>60</sub>	6.55	10.70	7.22	8.16
$N_{120}^{00}$	6.82	11.53	7.80	8.72
LSD <sub>0.05</sub>	1.85	2.47	2.37	2.23

Nitrogen fertilisers increased biomass yield. Fresh biomass yield was significantly increased by the 120 kg ha<sup>-1</sup> rate, and that of dry biomass by both rates; however, the differences

between different rates were non-significant. In the third year, *Miscanthus* productivity was, on average, 82% higher than in the second year and dry biomass yield amounted to  $11.5 \text{ t ha}^{-1}$ . Nitrogen fertilisers that year did not give any significant increase in biomass yield. After a more severe winter in the fourth year of growth a marked reduction occurred in *Miscanthus* dry biomass yield. This reduction resulted not only from plant thinning but also from a lower number of shoots per plant (13.2–16.3). In the second and third year of growth the number of shoots was higher, 18.2–23 per plant. Only the highest rate of nitrogen significantly increased biomass yield.

Calorific value of biomass of perennial grasses intended for combustion is directly dependent on fibre content. Biogas output depends on carbon and nitrogen contents and their ratio in the biomass (Kanapeckas *et al.*, 2011). Compared with leaves, the share of *Miscanthus* stems in the total biomass has a greater influence on fibre and carbon contents and determines energy characteristics of biomass. In the biomass of the third year of grow *Miscanthus* the content of neutral detergent fibre (NDF) increased (Table 2). The concentration of major elements (N, C, S) in *Miscanthus* biomass was more dependent on the stand age rather than on fertilisation.

Nitrogen	Chemical composition (g kg <sup>-1</sup> )										
fertilization	Ν	С	S	NDF	Ash	Lignin					
Second year of growth/ Third year of growth											
N <sub>0</sub>	10.3/6.8	462/472	2.7/1.4	793/817	53.8/34.5	84.7/109					
N <sub>60</sub>	13.7/5.8	472/472	1.8/1.4	808/811	51.9/34.1	87.6/982					
N <sub>120</sub>	12.5/5.4	464/478	1.9/1.2	795/837	54.1/24.2	70.3/114					

Table 2. Chemical composition of Miscanthus x giganteus aboveground biomass

# Conclusions

The assessment of the *Miscanthus* hybrid under a colder climate zone in Lithuania was valuable both from the practical and theoretical viewpoints. Persistence of the *Miscanthus* plants introduced to Lithuania from Austria was relatively good over the four growing seasons and three wintering periods. Nitrogen fertilisers gave a significant increase in *Miscanthus* biomass yield but not in each of the experimental years. The hybrid *Miscanthus* x *giganteus* can be recommended for growing in Lithuania for bioenergy purposes.

# Acknowledgements

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# References

Christian D.G., Riche A.B. and Yates N.E. (2008) Growth, yield and mineral content of *Miscanthus* x *giganteus* grown as a biofuel for 14 successive harvests. *Industrial Crops and Products* 28, 320–327.

Heaton E., Dohleman G.F. and Long S.P. (2008) Meeting us biofuel goals with less land: the potential of *Miscanthus. Global Change Biology* 14, 9, 2000–2014.

Kanapeckas J., Lemeziene N., Butkute B. and Stukonis V. (2011) Evaluation of tall fescue (*Festuca arun-dinacea* Schreb.) varieties and wild ecotypes as feedstock for biogas production. *Zemdirbyste*=Agriculture 98, 2, 149–156.

Lewandowski I., Clifton-Brown J.C., Scurlock J.M.O. and Huisman W. (2000) *Miscanthus*: European experience with a novel energy crop. *Biomass and Bioenergy* 19, 209–227.

Pappas I., Koukoura Z., Goulas Ch., Kiparissides C. and Tananaki Ch. (2009) Perennial herbaceous species biomass as potential bioenergy feedstock. *Grassland Science in Europe* 15, 425–427.

# Comparison of dry matter yield of lignocellulosic perennial energy crops in a long-term Belgian field experiment

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# Abstract

In pursuit of a sustainable production of biomass for energy, a comparative trial was installed in Merelbeke (Flanders, Belgium) with six perennial lignocellulosic species: *Miscanthus x giganteus*, *M. sinensis*, *Panicum virgatum*, *Phalaris arundinacea*, *Phragmites australis*, and willow managed in a short rotation coppice scheme. This field trial was set up in May 2007 and has been managed under low input conditions (no fertilizer and herbicides). In March 2011, the trial was harvested for the fourth time. During establishment in 2007 and 2008, the dry matter (DM) yield was low and varied in spring 2008 between 0.5 and 5.6 t DM ha<sup>-1</sup>. In the third and fourth year, yield increased and in spring 2011, DMY varied between 4.2 and 19.81 t DM ha<sup>-1</sup>, with *M. x giganteus* having the highest productivity. Cell wall components (NDF, ADF, ADL) were determined for all harvests in view of their use as feedstock in second generation bio-ethanol production.

Keywords: biomass yield, composition, miscanthus, switchgrass, willow

# Introduction

The increasing worldwide energy consumption, the concerns about environment protection, and the decrease of the world reserves of fossil energy have increased the demand for alternative energy sources. Bio-ethanol is one of the possible solutions. Second generation bio-ethanol is generated from lignocellulosic biomass (Somerville, 2007). This biomass can be obtained from agricultural or forestry waste but also from dedicated energy crops. Important characteristics of an energy crop are high yield, low input requirements and perenniality. In addition, quality of biomass will be an important determinant of the potential of a crop as raw material for bio-ethanol production. Good-quality biomass, when focusing on bio-ethanol production, should contain a large amount of polysaccharides which can be released and hydrolyzed into monosaccharides. The large amounts of cellulose and hemicelluloses contained in plant cell walls are the sugar resources for bio-ethanol production, whilst lignin constitutes a limiting factor in the conversion process (Keating *et al.*, 2006).

Limited information is available on the yield potential and the advantages and disadvantages of dedicated energy crops under Belgian conditions. A field trial was therefore set-up with the main purpose to compare the biomass yield and biomass quality of *Panicum virgatum* (Switchgrass), *Phalaris arundinacea* (red canary grass), *Miscanthus* spp. (elephant grass), *Phragmites australis* (common reed) and *Salix* spp. (willow). Here we report the results of the first four years after establishment of the respective 5 perennial crops.

# Materials and methods

In May 2007, a field trial was established including two cultivars of *Panicum virgatum* (Cave in Rock and Kanlow), two cultivars of *Phalaris arundinacea* (Bamse and Palaton), two *Miscanthus* spp. clones (*Miscanthus x giganteus* – Nl and *Miscanthus sinensis* – Goliath), one *Salix* spp. clone and one *Phragmites australis* cultivar. The trial was arranged

in a randomized block design with three replicates. Each crop was sown or planted at the density recommended for large scale plantations in plots of 28 m<sup>-2</sup>. *Panicum virgatum, P. arundinacea* and *P. australis* were sown at a density of 1000 seeds/m<sup>-2</sup>. *Miscanthus* rhizomes were planted at a density of 2 m<sup>-2</sup>. For *Salix*, cuttings were planted following a typical short rotation coppice design. After establishment (April-May 2007), weeds were controlled with herbicides. No fertilization was applied. The different crops were harvested once a year in February-March, except for willow which was harvested once in three years. Fresh and dry matter (DM) yields were determined. Cell wall composition of the harvested material was determined according to the NDF, ADF and ADL method described by Van Soest (1991). One-way ANOVA was used to identify significant differences between crops and years. The Duncan's multiple range test was used as post-hoc analysis to identify the differences between the groups. All analyses were carried out in SPSS 17 (IBM, Armonk, USA).

#### **Results and discussion**

Biomass production was low (between 0.5 and 5.6 t DM ha<sup>-1</sup>) in the year of establishment but increased significantly in the second year (between 3.7 and 15.4 t DM ha<sup>-1</sup>) (Table 1). In the third year *Miscanthus* produced 25.7 t DM ha<sup>-1</sup> and was the highest yielding crop. Dry matter yield of reed canary grass decreased in the third year, producing less than 1 t DM ha<sup>-1</sup>. This crop is not suited to being harvested once a year in early spring as it had already senescenced in July, having a negative effect on persistency. The other perennial energy crops require two years of establishment before the maximum productivity is reached and *Miscanthus x giganteus* is the most promising crop with a yield of approximately 20 t DM ha<sup>-1</sup>.

	DMY2	DMY2008		DMY2009		2010	DMY2011	
Crops	(t ha	l <sup>-1</sup> )	1) (t ha-1)		(t ha-1)		(t ha-1)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Reed canary grass cv. Palaton	4.00 <sup>c</sup>	0.62	8.59 <sup>b</sup>	1.25	0.833ª	0.57	_	
Reed canary grass cv. Bamse	5.64 <sup>d</sup>	1.10	9.47 <sup>b</sup>	1.42	0.76ª	0.33	_	
Switchgrass cv. CIR	2.52 <sup>b</sup>	0.63	12.69°	1.10	8.75°	4.77	9.54°	0.11
Switchgrass cv. Kanlow	2.58 <sup>b</sup>	0.53	12.90°	2.58	15.94 <sup>b</sup>	0.97	13.96ª	0.81
Miscanthus x giganteus	3.31 <sup>b.c</sup>	0.86	15.44 <sup>d</sup>	0.68	25.72 <sup>d</sup>	1.25	19.80 <sup>d</sup>	1.55
Miscanthus sinensis cv. Goliath	0.49ª	0.18	4.09 <sup>a</sup>	0.55	13.98 <sup>b</sup>	3.98	14.27ª	4.04
Phragmites australis	0.92ª	0.25	3.72ª	0.62	3.62ª	1.38	4.15 <sup>b</sup>	0.68
Willow*	_		-		12.50 <sup>b,c</sup>	3.22	-	

Table 1. Dry matter yield of 7 crops in spring 2008, 2009, 2010 and 2011

<sup>a,b,c,d,e</sup> homogeneous subsets by use of Duncan's multiple range test (P < 0.05).

\*Willow was harvested once in 3 year. DMY in 2010 is calculated on a yearly basis.

In view of bio-ethanol production, the cell wall composition is of major importance. The cell wall fraction is a measure of the total sugar content entrapped in the cell wall, and lignin content reflects the accessibility of the cell wall for lignocellulolytic enzymes (Berlin *et al.*, 2006). In Table 2, significant differences in NDF content between the studied crops were identified. Reed canary grass, reed and switchgrass had the lowest NDF content, willow had intermediate NDF content and *Miscanthus* has the highest NDF content. Lignin content was the highest in willow and *Mischanthus* (ADL = 13.4% and 12%, respectively). The latter two crops have the highest potential for bio-ethanol production given the highest of the studied crops and should be taken with care in view of bio-ethanol prodution.

	NE	)F	AD	F	AD	)L	Re	st	
Crops	(% of ADW)		(% of A	ADW)	(% of A	ADW)	(% of A	ADW)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Reed Canary Grass cv. Palaton	33.4 °±0.6		34.4 °±0.7		7.8 °±0.4		24.4 °± 1.2		
Reed Canary Grass cv. Bamse	32.7 <sup>a</sup> ±1.7		35.3 °	35.3 °±0.9		7.9 <sup>a</sup> ±0.8		24.1 °±1.6	
Switchgrass cv. CIR	44.1 °±0.7		31.8 <sup>a,b</sup> ±0.6		10.0 °±0.6		14.1 <sup>b</sup> ±0.7		
Switchgrass cv. Kanlow	43.8 <sup>b,0</sup>	°±0.6	33.1 <sup>b</sup> ±0.3		8.2 <sup>a,b</sup> ±0.3		14.9 <sup>b</sup> ±0.6		
Miscanthus x giganteus	49.3 f	±1.4	27.0 °±1.5		$12.0^{d} \pm 1.2$		11.7 <sup>a</sup> ±1.2		
<i>Miscanthus sinensis</i> cv. Go- liath	46.4 °	±0.5	32.8 <sup>a,b</sup> ±0.3		8.7 <sup>a,b</sup> ±0.4		12.0 <sup>a</sup> ±0.2		
Willow	39.1 <sup>d</sup>	±0.7	14.7 <sup>d</sup>	14.7 <sup>d</sup> ±0.5		13.4 °±0.5		32.8 °±1.9	
Phragmites australis	42.4 <sup>b</sup>	±0.4	31.5 <sup>a</sup> ±0.4		9.2 <sup>b,c</sup> ±0.6		16.9 <sup>d</sup> ±0.8		

Table 2. Cellulose, hemicellulose, lignin and the non-cell wall fraction of crops, as percentage of absolute dry weight (ADW) in harvest 2010

<sup>a,b,c,d,e</sup> homogeneous subsets by use of Duncan's multiple range test (P < 0.05).

#### Conclusions

These four years' results indicate that *Miscanthus x giganteus* might be the most productive lignocellulosic crop under Belgian conditions. Reed canary grass was the most productive crop in the first year after establishment, but was not able to persist under the tested management of one cut per year. Differences were identified in cell wall content and composition, indicating that willow and *Miscanthus x giganteus* were crops with the highest NDF contents and also the highest ADL content.

#### References

Berlin A., Balakshin M., Gilkes N., Kadla J., Maximenko V., Kubo S. and Saddler J. (2006) Inhibition of cellulase, xylanase and β-glucosidase acitivities by softwood lignin preparations. *Journal of Biotechnology* 125, 198–209.

Keating J.D., Panganiban C. and Mansfield S.D. (2006) Tolerance and adaptation of ethanologenic yeasts to lignocellulosic inhibitory compounds. *Biotechnology and Bioengineering* 93, 1196–1206.

Somerville C. (2007) Biofuels. Current Biology 17, R115-R119.

Van Soest P.J., Robertson J.D. and Lewis B.A. (1991) Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharide in relation to animal nutrition. *Journal of Dairy Science* 74, 3583–3597.

# Session 3.2.

# Additional provisioning services of grassland

# Small-leaved white clover (*Trifolium repens* L. form *microphyllum*) as a component of a lawn mixture

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# Abstract

White clover is often regarded as a weed in turf, but it can provide aesthetic and environmental advantages. Turf quality of grasses by themselves, and in mixture with a small-leaved white clover, was compared on light soil over 3 years. The experiment was a factorial design with N-fertilizer rates of 0, 40, 80, 120, 160 and 200 kg ha<sup>-1</sup>, and mowing heights of 2.5 and 5.5 cm. The assessment of the lawn was carried out using a 9° scale. Under the influence of N-fertilization, the proportion of clover in the sward decreased from about 50% to 5%. Introduction of clover to the grass mixture caused an improvement (mostly by 1°) in green intensity, sward compactness, plant health and the general aspect of the lawn. Low cutting resulted in a 6% increase in the proportion of clover in the lawn sward, improved green intensity, and health of plants. Increasing nitrogen fertilization up to 160 kg ha<sup>-1</sup> improved the quality of lawn without clover but had no effect on the lawn with white clover. The addition of small-leaved white clover to a typical lawn mixture improved turf quality while eliminating need for N-fertilizer.

Keywords: lawn aesthetic quality, lawn fertilization, green intensity, cutting height, small-leaved white clover

#### Introduction

White clover (Trifolium repens L.) occurring in lawns is usually treated as a weed. This species, however, and especially its small-leaved form (microphyllum), has properties that can improve the aesthetic quality of lawns, particularly those utilized extensively. Apart from the ability to fix atmospheric nitrogen, white clover is characterized by its ability to form a dense turf quickly, survive periodic droughts and maintain the green colour of leaves in the summer season. Lawns with mixtures that are composed only of grasses often turn yellow and wither in summer. Maintaining the attractive appearance of the sward requires watering and applying high nitrogen fertilization. This not only increases the maintenance costs but also stimulates nitrate leaching and, consequently, has a negative impact on the soil environment. Low and frequent cutting of grasses stimulates tillering and consequently improves sward compactness and the aesthetic quality of the lawn. Higher cutting leads to thinning of the lawn, and thus it may create an area for white clover development. However, the proportion of clover in the species composition of the lawn mixture may be affected by nitrogen fertilization. The aim of this study was to estimate the level of nitrogen fertilization and cutting height that provide the best ground cover and allow retention of green colour and good general assessment of the sward.

# Materials and methods

The field experiment was carried out on light soil near Bydgoszcz, Poland from 2007 to 2010 (establishment year + 3 production years). The quality of sward established was based on a mixture composed of grasses only (*Lolium perenne*, *Festuca rubra* and *Poa pratensis*) and this was compared with a mixture of the same grasses with 5% mass fraction of the

small-leaved white clover cv. Rivendel in the seed material. The experiment was a factorial design with N-fertilizer rates of 0, 40, 80, 120, 160 and 200 kg ha<sup>-1</sup>, and mowing heights of 5.5 and 2.5 cm. The experiment was conducted in 3 replications and the plot area was 2 m<sup>2</sup>. Cultivation treatments were carried out in accordance with recommendations for extensive lawn utilization given by Domański (1998). Nitrogen fertilizer was split-applied to each treatment four times during each growing season. The sward was cut when the plants reached a height of 10 cm and clippings were removed from the plots. The number of cuts ranged from 12 to 16 per year. Irrigation was not applied. The total precipitation in the period from March to the end of October was: 2008 - 381, 2009 - 423, 2010 - 508 mm. Assessments were carried out three times in each growing season: in the second half of April,

at the end of July and at the beginning of October. The proportion of clover in the sward was evaluated as %. Green intensity, sward compactness, plant health and the general aspect were assessed visually, using a 9-degree scale (1 = the poorest state, 9 = the best state).

The results presented in this study are a synthesis from the three production years. The analysis of variance was applied to assess the significance of the proportion of clover in the sward, and the ANOVA rank Kruskal-Wallis test was used for the assessment of features on a 9-degree scale. The results from these periods are presented in the tables for chosen effects. The results of assessments on a 9-degree scale are presented in tables as medians. The program Statistica 7.0 was used.

#### **Results and discussion**

Introduction of small-leaved clover to a typical grass mixture resulted in an improvement (mainly by 1°) of green intensity, sward compactness, health of plants and the general aspect (Table 1). Green intensity and the general aspect were assessed higher not only in the spring period but also in the whole growing season. An improvement of sward compactness was obtained in summer, and of the plant health state in autumn. Table 1 also demonstrates an effect of cutting height on lawn features irrespective of the mixture composition, since there was no difference in response of mixture without clover and with clover to a diversification of cutting height. Contrary to expectations, cutting at a height of 5.5 cm did not cause an increase in proportion of clover in the lawn sward. At low cutting, the proportion of clover was on average 6% higher than with cutting at a height of 5.5 cm. As a result, cutting at a height of 2.5 cm improved the green intensity in spring and over the entire the growing season, whereas an improvement in plant health state took place in the autumn period. Increasing N-fertilization increased green intensity, sward compactness and the general aspect of the sward without clover (Table 2). Successive rates of N-fertilization significantly decreased the proportion of clover in the lawn sward. In lawns with clover, green intensity, sward compactness and the general aspect depended both on the presence of clover and mineral N availability for grasses. Similar results are described by Sincik and Acikgoz (2007). As a result, the green intensity and general aspect of the sward with clover was affected little by fertilization with nitrogen. The colour of the lawn with clover was always more green than that of the lawn without clover, but as nitrogen rates increased, the differences in assessment of both types of lawn decreased systematically - from 2 to 0.5 degrees (Table 2). In the summer season comparable sward compactness of the lawn with clover and without clover was obtained when N was applied at 160 kg ha<sup>-1</sup>, but the ideally compact sward was obtained in the lawn with clover fertilized with 120 kg ha-1 N. Particularly large differences in assessment of the general aspect between the lawn with clover and without clover were recorded in the summer period, where nitrogen fertilization ranged from 0 to 80 kg ha<sup>-1</sup>. The general aspect of the mixture without clover was equal to the mixture with clover only at N- fertilization at rates 160 and 200 kg ha<sup>-1</sup>.

It was not observed that introducing clover to the grass mixture caused deterioration in any of the assessed visual features of the sward. Data over 3 years show no benefit of N application to grass-clover mixtures if clippings are removed; however the long-term performance is unknown.

	·	U	0				
Denied of concernent	Mixture composition			Cutting height			
Period of assessment	without clover	r with clover	significance	5.5 cm	2.5 cm	significance	
Proportion of clover in sward (%)							
Summer	_ `	27.4	_	24.2	30.6	*	
Whole growing season	_	25.3	_	22.6	28.1	*	
Green intensity							
Spring	6.0	7.0	ns	5.5	6.0	*	
Whole growing season	6.0	7.0	**	6.0	7.0	*	
		Sward compa	ctness				
Summer	7.5	8.0	*	7.5	7.5	ns	
		Plant hea	lth				
Autumn	7.0	8.0	*	7.0	8.0	*	
		General as	pect				
Spring	5.5	6.0	**	5.5	6.0	ns	
Whole growing season	6.0	7.0	**	6.0	6.0	ns	
ne not significant *	significant at P	< 0.05 **	ignificant at P	< 0.01			

Table 1. Effect of mixture composition and cutting height on lawn assessment

ns – not significant, \* – significant at P < 0.05, \*\* – significant at P < 0.01.

Table 2. Effect of nitrogen fertilization on lawn assessment depending on mixture composition

	-							
Deriod of assassment Mixture composition			Nitrogen rate (kg·ha <sup>-1</sup> )					
Period of assessment	Mixture composition	0	40	80	120	160	200	cance
Proportion of clover in sward (%)								
Whole growing	with alaryan	18.0	40.4	28.0	10.2	0.7	57	**
season	with clover	48.9	40.4	28.0	19.5	9.7	5.7	
Green intensity								
Whole growing	without clover	5.0	5.5	6.0	6.0	6.5	6.5	**
season	with clover	7.0	7.0	7.0	7.0	7.0	7.0	ns
	Sward	compa	ctness					
	without clover	5.0	6.0	7.0	8.0	8.0	8.0	**
Summer	with clover	8.0	8.0	8.0	9.0	8.0	8.0	ns
General aspect								
Summer	without clover	3.5	4.0	5.5	6.0	7.0	8.0	**
Summer	with clover	8.0	7.0	7.0	7.0	7.5	8.0	ns
Whole growing	without clover	5.0	5.0	6.0	6.0	7.0	7.0	**
season	with clover	7.0	7.0	7.0	7.0	7.0	7.0	ns

ns – not significant, \* – significant at P < 0.05, \*\* – significant at P < 0.01.

#### Conclusions

The addition of small-leaved white clover to a typical lawn mixture improved turf quality of lawns grown on light soils with no irrigation. The implications are substantial savings associated with elimination of N fertilizer use, and elimination of the risk of environmental contamination by N fertilizer leaching or runoff. However, maintenance of lawn with small-leaved white clover requires low cutting – at 2.5 cm.

#### References

Domański P. (1998) Methods for examination of the economic value of a variety of field crops. Agricultural crops. Sward (lawn) grasses, COBORU, Słupia Wielka, Poland, 38 pp. (in Polish).

Sincik M. and Acikgoz E. (2007) Effects of white clover inclusion on turf characteristics, nitrogen fixation, and nitrogen transfer from white clover to grass species in turf mixtures. *Communications in Soil Science and Plant Analysis* 38, 1861–1877.

# The effect of management of cut plant residues on soil moisture content of grassland swards

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# Abstract

The aim of this study was to assess the impact of returned grass material on soil moisture in turfgrass and grass-clover swards at different fertilization rates. The experiment was carried out during 2006–2007. The soil was sampled at the 0–5 and 5–20 cm soil layers by manual coring and gravimetric moisture content (%) of the soil samples was calculated on oven dry weight basis. The factors of the experiment were: (i) sward type; (ii) residue treatment; (iii) fertilizer treatment. The cut plant residues returned to the plot influenced soil moisture content only in the year when the vegetation period was more humid. The influence was statistically significant only in 0–5 cm soil layer in both swards. Fertilization had a negative effect on the soil moisture in turfgrass sward, which was associated with an increase in sward productivity. The fertilization impact on soil moisture content of grass-clover swards was not significant.

Keywords: grass, soil moisture, sward productivity, mowing

# Introduction

The availability of water is one of the most important factors that can limit grassland productivity. Plant residues left on the soil surface strongly influence radiation balance and reduce the rate of evaporation from the soil (Wilhelm *et al.*, 2004), which in turn increases the soil moisture (water) content (Döring *et al.*, 2005). The plant residues that left to the surface of the grassland to decompose are the products mainly from turfgrass swards and set-aside grasslands. The objective of the study was to investigate how plant residues from different swards and various sward management strategies (fertilization, frequency of mowing) influence the content of soil moisture.

# Materials and methods

The field experiment was carried out at the Experimental Station of the Estonian University of Life Sciences in Tartu. The soil of the experimental field was a sandy loam *Stagnic Luvisol* according WRB classification (FAO, 2006). The swards had been established in 2003 with turfgrass mixture (*Festuca rubra rubra* and *Poa pratensis*) and with grass-clover mixture (*Phleum pratense, Lolium perenne* and *Trifolium repens*). The soil moisture content was measured twice a month from May to September in both experimental years. The soil was sampled at the 0–5 cm and 0–20 cm soil layer by manual coring. Gravimetric moisture content (%) of the soil samples was calculated on oven dry weight basis. The factors of the experiment were: (i) sward type: turfgrass sward (*Festuca rubra rubra* and *Poa pratensis*) and grass-clover sward (*Phleum pratense, Lolium perenne* and *Trifolium repens*); (ii) residues treatment: the cut plant residues were returned (RRT) to the plots or removed (RRM) from the plots after the mowing; (iii) fertilizer treatments:  $N_0P_0K_0$  (N0),  $N_{so}P_{11}K_{4s}$  (N80),

 $N_{160}P_{22}K_{96}$  (N160) and  $N_{400}P_{56}K_{240}$  (N400) kg ha<sup>-1</sup> for turfgrass sward and  $N_0P_0K_0$  (N0) and  $N_{80}P_{26}K_{50}$  (N80) kg ha<sup>-1</sup> for grass-clover sward. The mowing frequency was 14–15 times for turfgrass sward and 3–4 times for grass-clover sward per growing season. A rotary mulching lawn mower with a bag attachment was used for cutting the plots. The material was removed from the bag after every cutting and weighed for the dry matter (DM) yield measurement. After the weighing procedure, cut plant materials were either returned (RRT) to the plots or removed (RRM) from the plots. Average air temperature from May to September in year 2006 was 15.8°C and in 2007 15.6°C. There was less rain and longer periods of high temperature (daily air temperatures above 25°C) from end of June to July in 2006. Average air humidity varied from 81.6% (in 2006) to 85.5% (in 2007). Precipitation was lower in 2006 (150 mm) than in 2007 (172 mm). In 2007 the rainfall was evenly distributed during growing period. ANOVA was used to test the effect of fertilization and cut plant residues management on swards productivity and soil moisture. The Fisher's LSD test was used to distinguish means. All calculations were carried out using the statistical package Statistica 9 (StatSoft Inc.).

#### **Results and discussion**

Average soil moisture was significantly lower in 2006 than in 2007 (Table 1), and it was influenced by the weather conditions in 2006. Latter conditions decreased the soil moisture content in that period up to 5% (data not shown).

Plant residues did not influence soil moisture in either of the investigated soil layers (0-5 cm and 0-20 cm) in 2006. Most likely this was due to the amount of plant residues left on the sward surface being insufficient to show a positive effect on soil moisture content. A similar conclusion was drawn by Döring *et al.* (2005).

	2006			2007				
Fertilization rate,	$RRM^1$	RRT <sup>2</sup>	RRM	RRT	RRM	RRT	RRM	RRT
kg N ha <sup>-1</sup>	0–5	cm	5-20	) cm	0–5	cm	5-20	cm
-			1	Turfgrass	sward			
N0	11.4 <sup>a3C4</sup>	11.6 <sup>aC</sup>	11.3 <sup>aC</sup>	11.5 <sup>aC</sup>	14.3 <sup>aC</sup>	14.9 <sup>bC</sup>	14.6ªD	14.8 <sup>aC</sup>
N80	10.4 <sup>bB</sup>	10.3 <sup>aB</sup>	10.2 <sup>aB</sup>	10.3 <sup>aB</sup>	13.0 <sup>aB</sup>	14.3 <sup>bC</sup>	12.8°C	13.1 <sup>aB</sup>
N160	9.7 <sup>aA</sup>	9.4 <sup>aA</sup>	9.6 <sup>aA</sup>	9.6 <sup>aA</sup>	12.0ªA	13.4 <sup>bB</sup>	12.1ªB	11.1 <sup>aA</sup>
N400	9.3ªA	9.3ªA	9.2ªA	9.4 <sup>aA</sup>	11.9 <sup>aA</sup>	11.9 <sup>aA</sup>	11.1 <sup>aA</sup>	11.0 <sup>aA</sup>
			G	rass-clove	er sward			
N0	10.2ªA	10.5 <sup>aA</sup>	9.1 <sup>aA</sup>	9.3ªA	13.6ªA	15.7 <sup>aA</sup>	12.4 <sup>aB</sup>	12.9 <sup>aB</sup>
N80	9.8 <sup>aA</sup>	10.4 <sup>aA</sup>	9.1ªA	9.5ªA	13.4ªA	15.1 <sup>bA</sup>	11.8 <sup>aA</sup>	12.1ªA

Table 1. Soil moisture content (% dry matter basis) depending on fertilization and management of cut plant residues in 2006 and 2007

<sup>1</sup>RRM – plant residues were removed from the plots; <sup>2</sup>RRT – plant residues were returned to the plots; <sup>3</sup>Small letters within each row indicate a significant effect (P < 0.05) of returning plant residues on the soil moisture in different soil layer; <sup>4</sup>Capital letters within column indicate a significant effect (P < 0.05) of fertilization on the soil moisture.

The effect of returning plant residues to the plots had a statistically significant impact on soil moisture content in surface soil layer. No effect was observed in the lower soil layer (5–20 cm). Plant residue impact was significantly higher in the grass-clover sward, increasing soil moisture content 1.7–2.1%, compared to the turfgrass sward. Enhanced effect of grass-clover sward plant residues could be due to mowing frequency. Grass-clover sward was mown 3–4 times during the growing period and consequently more plant material was left to decompose on the surface. As a result, the impact of plant residues could have lasted longer preventing soil moisture decrease by evaporation (Wilhelm *et al.*, 2004). Less plant material was left to decompose on the surface of the turfgrass sward after each mowing, hence decreasing the impact of plant residues.

Sward productivity had significant impact on soil moisture content, especially on the turfgrass sward. Turfgrass sward soil moisture content was the lowest where the DM yield was highest (fertilization treatment N400) (Table 1, 2). Fertilization had a smaller effect on grass-clover sward yield; therefore, the soil moisture content did not vary between different fertilization treatments as much. Results clearly indicate that soil moisture content was influenced by plant water uptake – water-use increases with higher yields plants. These results concur with the findings of previous studies (De Jong and MacDonald, 1975; Raave *et al.*, 2009).

Table 2. DM yields (kg ha<sup>-1</sup>) of the swards depending on fertilization and management of cut plant residues in 2006 and 2007

	20	006	20	07			
Fertilization rate,	$RRM^1$	RRT <sup>2</sup>	RRM	RRT			
kg N ha <sup>-1</sup>	Turfgrass sward						
NO	1294 <sup>a3A4</sup>	854 <sup>aA</sup>	1144 <sup>aA</sup>	990 <sup>aA</sup>			
N80	1760 <sup>aB</sup>	1730 <sup>aB</sup>	2317 <sup>aB</sup>	2511 <sup>aB</sup>			
N160	2197 <sup>aC</sup>	2341 <sup>aC</sup>	2903 <sup>aC</sup>	3541 <sup>bC</sup>			
N400	2979 <sup>aD</sup>	2755ªD	5155 <sup>aD</sup>	5481 <sup>aD</sup>			
		Grass-c	lover sward				
N0	4093 <sup>aA</sup>	6174ªA	4470 <sup>aA</sup>	7790 <sup>aA</sup>			
N80	4628 <sup>aB</sup>	6295 <sup>aA</sup>	5210 <sup>aB</sup>	7720 <sup>aA</sup>			

<sup>1</sup>RRM – plant residues were removed from the plots; <sup>2</sup>RRT – plant residues were returned to the plots; <sup>3</sup>Small letters within each row indicate a significant effect (P < 0.05) of returning plant residues on the sward field; <sup>4</sup>Capital letters within column indicate a significant effect (P < 0.05) of fertilization on the sward yield.

#### Conclusions

The sward soil moisture content depended on the quantity of returned plant residues in a positive relationship: greater amounts of returned plant residues increased soil moisture content. The amount of returned plant residues depended on the sward fertilization treatment and frequency of mowing.

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# References

De Jong E. and MacDonald K.B. (1975) The soil moisture regime under native grassland. *Geoderma* 14, 207–221.

Döring T.F., Brandt M., Hesz J., Finckh M.R. and Saucke H. (2005) Effects of straw mulch on soil nitrate dynamics, weeds, yield and soil erosion in organically grown potatoes. *Field Crops Research* 94, 238–249. FAO, IUSS and ISRIC (2006) *World Reference Base for Soil Resources 2006*, Second Edition. World Soil Resources Report 103, FAO, Rome, 128 pp.

Raave H., Kauer K., Noormets M., Selge A. and Viiralt R. (2010) Nitrogen and potassium leaching from grassland soil depending on applied fertilizer type and rate and sward botanical composition. *Grassland Science in Europe* 15, 1058–1060.

Wilhelm W.W., Johnson J.M.F., Hatfield J.L., Voorheesd W.B. and Lindene D.R. (2004) Crop and soil productivity response to corn residue removal: a literature review. *Agronomy Journal* 96, 1–17.

# Seed multiplication of *Trifolium alpinum*: crop persistency and harvesting methods

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## Abstract

The seed multiplication of ecotypes of perennial alpine plants is often characterised by considerable labour costs and intensive maintenance care. The crop persistency is of great importance in determining the economic feasibility of cultivation. The seed of *Trifolium alpinum*, a desirable seed mixture component for the revegetation of strongly acid soils at high altitude in the Alps, can be successfully propagated, but less is known about the crop persistency. In a six-year field trial, plant cover, several yield components and seed yield were surveyed over time. Two harvesting methods (manual harvest, mechanised harvest) were investigated as well. The yield considerably increased in the first two harvest seasons, but then abruptly dropped to a very low level, mainly due to low infructescence density and a high proportion of empty florets, while plant cover remained constant. The mechanised harvest led to slightly higer thousand seed weight and lower seed yield. It is concluded that the seed production of *Trifolium alpinum* is reasonably limited to two harvest years. The mechanical harvest, despite a 20% lower seed yield, seems to be feasible in order to reduce the cultivation costs and, in turn, the seed price.

Keywords: *Trifolium alpinum*, ecological restoration, seed multiplication, crop persistency, harvesting method

#### Introduction

*Trifolium alpinum* is a desirable seed mixture component for the ecological restoration of strongly acidic soils at high altitude in the Alps (Krautzer *et al.*, 2004). However, because of low potential growth rate and phytosanitary problems, its seed multiplication has repeatedly proven to be a difficult task to accomplish (Peratoner *et al.*, 2007). This results in considerable production costs, due among all to crop establishment with transplants. The crop persistency is therefore of great importance in determining the economic feasibility of the cultivation over years. In this respect, a mechanised seed harvest would be desirable as well. However, less is known about crop persistency and mechanised harvest of *Trifolium alpinum*. For this reason, a six-year field experiment was conducted to elucidate these aspects.

#### Materials and methods

The field experiment was located in the upper montane belt at the nursery garden Prettau (1,600 m a.s.l., Ahr valley, South Tyrol, Italy). Container-grown plants of *Trifolium alpinum* obtained by seed of a locally collected ecotype were used to establish the field trial as described by Peratoner *et al.* (2009). Two factors were investigated: the inoculation with original topsoil (present/absent) obtained at the original site of the seed collection and two different harvesting methods (manual/mechanical). Mechanised harvest was performed starting from the second harvest year (third growing season) by means of a Minicut 3000 (Hortiplus, Pfäffikon, CH), a motor-powered, portable harvesting device, provided with a 40 cm-wide mowing bar and a blower driving the harvested plant material into a collecting

bag. Plots (1.35 m<sup>2</sup>) were arranged in a split-plot design with four replications, with the harvesting method as split factor. Prior to each harvest, projective cover of the crop plant, infructescence density and percentage of empty florets (3 samples of 100 florets per plot) were determined in a permanent sampling area of 0.25 m<sup>2</sup>. The harvested plant material was dried at 30°C for three days before seed cleaning. Thousand seed weight (TSW) was determined according to ISTA (1996) in three replicates per plot. Data were statistically analysed by means of a mixed model. Inoculation, harvesting method, harvest year, block and all their interactions were treated as fixed effects, while the interaction block  $\times$  inoculation was regarded as a random effect. The harvest year was considered to be a repeated factor with the plots being the subject of repeated measurements. Multiple comparisons were performed by LSD. Data failing even after transformation to fulfil requirements of normal distribution of residuals and variance homogeneity were subjected to nonparametric tests (Mann-Whitney-U-test for independent observations or Friedman-test and multiple comparisons by the Wilcoxon-Wilcox-test for repeated measures). All tests were carried out at  $\alpha = 0.05$ . Data from the first harvest year (2007) were not included in the statistical analysis because the factor harvesting method was first applied in 2008.

#### **Results and discussion**

All investigated traits were significantly affected by the harvest year, with the exception of plant cover, which did not significantly change between 2008 and 2011 (Table 1). In 2009, the third harvest year, infructescence density and seed yield abruptly dropped and remained on a very low level, with minor fluctuations, until the end of the experiment. Also, the density of unripe infructescences, as well as their proportion, increased over time. Moreover, the percentage of empty florets, ranging between 24 and 62% in 2009 to 2011, further contributed to depress the seed yield. The proportion of empty florets was already high in 2008, but was overcompensated by the high infructescence density. It is notable that TSW reached its lowest value in 2008, in which the seed yield peaked. These results suggest that despite sufficient plant persistency, as shown by the cover data, only two harvest seasons are economically meaningful. These findings are in accordance with results concerning other alpine species, and especially alpine legumes, which were found to allow one or two harvest years (Krautzer *et al.*, 2004).

Trait –		Harvest year					
		2008	2009	2010	2011	of effect	
Plant cover (%) #	84.1	46.3	51.1	52.1	45.7	0.265	
Infructescence density (No m <sup>-2</sup> ) <sup>†</sup>	611	589 a	32 °	138 <sup>b</sup>	84 <sup>b</sup>	< 0.001	
Density of unripe infructescences (No m <sup>-2</sup> ) <sup>†</sup>	269	1 <sup>b</sup>	3 <sup>b</sup>	19 <sup>a</sup>	24 a	< 0.001	
Unripe infructescences (%) *	36.6	0.4 <sup>b</sup>	14.9 <sup>ab</sup>	16.9 <sup>b</sup>	31.5 <sup>b</sup>	< 0.001	
Empty florets (%) **	n.a.	37.0 <sup>b</sup>	24.4 °	21.7 °	61.8 <sup>a</sup>	< 0.001	
TSW (g) **	4.68	3.89 <sup>b</sup>	4.76 <sup>a</sup>	4.85 <sup>a</sup>	4.43 ab	0.033	
Seed yield (kg ha <sup>-1</sup> ) *	158.8	201.6 <sup>a</sup>	2.3 bc	4.5 <sup>b</sup>	1.2 °	< 0.001	

Table 1. Changes over time of plant traits of *Trifolium alpinum*. 2007 data are summary values of two harvest dates. They are shown for reference only

n.a. - Not assessed.

\*\* Analysis with mixed model, untransformed data.

<sup>#</sup>Analysis with mixed model, arcsine-transformed data. Back-transformed means are shown.

<sup>†</sup>Analysis with mixed model, logarithm-transformed data. Back-transformed means are shown.

\* Friedman-test and multiple comparisons by Wilcoxon-Wilcox-test.

Plant cover was the only trait being affected by the interaction inoculation  $\times$  harvesting method (P = 0.019). Within the manually harvested treatment, inoculated plots exhibited higher plant cover than the uninoculated ones (58% vs. 43%). We suggest that the mechanised harvest hampered plant growth and thus plant cover, as aboveground plant biomass is periodically removed together with the infructescences, while differences between inoculated and uninoculated plots were detectable only in the manually harvested treatments, in which only the infructescences were removed and plant growth proceeded undisturbed. The effect of inoculation on plant cover, but not on other yield-related traits, confirms what already observed (Peratoner *et al.*, 2009).

Manual harv	vest Machanisad ha	P-value of effect
Trantour nur (	vest mechaniseu na	rvest
TSW (g) ** 4.44	4.54	0.046
Seed yield (kg ha <sup><math>-1</math></sup> y <sup><math>-1</math></sup> ) * 58.3	46.5	0.021

Table 2. Effect of harvesting method on TSW and seed yield

\*\* Analysis with mixed model, untransformed data.

\* Mann-Whitney-U-test.

Mechanised harvest resulted in slightly higher TSW and lower seed yield (Table 2). These facts indicate some kind of seed loss while operating with the harvesting device. Pods located near to the ground may be missed by the mowing bar or weakly developed seed may get lost during harvest operations. A direct detrimental effect of mowing on the plant performance would be the other possible explanation for changes in TSW and seed yield. In consideration that, in 2008, plants had no mowing disturbance prior to harvest, this is less supported by our data because of the lack of interaction between harvest year and harvesting method for TSW (P = 0.346). While the effect on TSW seems altogether of minor importance, seed yield is more severely affected by the mechanised harvest, resulting in a seed yield loss of about 20%.

#### Conclusions

Because of the extremely low seed yield from the third harvest year on, the seed production of *Trifolium alpinum* is reasonably limited to two harvest years only. Despite the lower seed yield caused by a mechanised harvest, the considerable reduction of labour costs let this harvesting method seem a viable alternative to the manual harvest.

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#### References

ISTA (1996) International rules for seed testing. Seed Science and Technology 24, 1-342.

Krautzer B., Graiss W. and Peratoner G. (2007) Seed production of subalpine and alpine leguminosae. In: Helgadottir Á. and Pötsch E.M. (eds). *Cost 852: quality legume-based forage systems for contrasting environments*, Federal Research and Education Centre Raumberg-Gumpenstein, Irdning, pp. 197–201. Krautzer B., Peratoner G. and Bozzo F. (2004) *Site specific grasses and herbs*. FAO Plant Production and

Protection Series 32. FAO, Rome, I, 112 pp.

Peratoner G., Joergensen R.M. and Spatz G. (2007) Growth of *Trifolium alpinum*: Effects of soil properties, symbionts and pathogens. *Ecological Engineering* 30, 349–355.

Peratoner G., Scherer E., Pramsohler M. and Gallmetzer W. (2009) Effect of inoculation, plant density and plant stand development on the seed multiplication of *Trifolium alpinum*. *Grassland Science in Europe* 14, 493–496.

# Effects of lime and sewage sludge on the relationship between tree cover and pasture production in a silvopastoral system on acid soil

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# Abstract

Agroforestry systems include sustainable land management techniques that are promoted by the EU (Council Regulation 1698/2005) and are considered by farmers of different European countries as a management system to implement. Silvopastoral production systems depend on forest species development and tree density established, but also on soil fertilization management practices, which may modify the tree-pasture relationship. *Pinus radiata* is broadly used in the Atlantic biogeographic region of Spain and it is a fast-growing species with a very dense canopy that could limit pasture production if planted at a high density. One of the main factors limiting pasture productivity on forestlands, especially at early plantation stages, is the low soil fertility, making it necessary to add products to overcome the acidity of the soils and the low nutrient availability. The aim of this study was to evaluate the relationship between tree cover and pasture production in a *Pinus radiata* stand developed on a very acid soil. The results obtained showed a negative linear relationship between litterfall biomass and pasture production and positive relationship between litterfall biomass and tree height.

Keywords: *Pinus radiata*, *Dactylis glomerata*, *Lolium perenne*, *Trifolium repens*, litterfall, tree height, light

# Introduction

Silvopasture is a form of agroforestry where trees are incorporated into managed pasture (Gordon et al., 2005). Competition for natural resources between trees and pasture species is one of the most important and studied interactions in silvopastoral systems (Chang and Mead, 2003). When a silvopastoral system is established in recently afforested land, the tree root system is usually not yet well developed, and the herbaceous plants-tree competition for water and nutrients can be higher than at later stages (Montero et. al., 2008). Moreover, just after establishment of tree plantation the pasture productivity is high, as the tree component does not reduce light reaching the soil, but usually pasture and trees compete for nutrients. Therefore, the maintenance of soil fertility through practices like liming or fertilization should be safeguarded to ensure adequate pasture growth without compromising tree development (Rigueiro et. al., 2009). Later, when the tree component has grown, tree shadow and litterfall biomass may limit the pasture production, so that the effect of fertilization on this production is smaller (Mosquera-Losada et al., 2011). The aim of this study was to evaluate the long-term effect of lime and two doses of sewage sludge at three different dates on the tree growth-pasture production relationship in a silvopastoral system established with Pinus radiata.

# Materials and methods

The experiment was located in the San Breixo Forest Community (Guitiriz, NW Spain). A plantation of *Pinus radiata* D. Don was established in 1998 at a density of 1667 trees ha<sup>-1</sup>

after harvesting a 30-year-old Pinus radiata D. Don stand. Scrubland was the main understory vegetation. When the tree stand was one year old, in October 1999, an experiment with a randomized block design was carried out in 39 (13 treatments  $\times$  3 replicates) experimental units of  $12 \times 8$  m<sup>2</sup>, each one consisting of 25 trees arranged in a 5×5 rectangle with a distance of 3 m between rows and 2 m between lines. Half of the plots were limed in October 1999 and half unlimed after the establishment of the experimental units. After liming, a seeds mixture of 25 kg ha<sup>-1</sup> Lolium perenne L. cv. Brigantia, 4 kg ha<sup>-1</sup> Trifolium repens L. cv. Huia of and 10 kg ha<sup>-1</sup> Dactylis glomerata L. var. Artabro was sown in all plots in early November 1999 after the first rains, as is usually performed in the area. Fertilization treatments consisted of two doses of sewage sludge of either 50 (L: low) and 100 (H: high) kg N ha-1, applied at three different dates (February (1), March (2) and April (3)) in limed and unlimed plots. Sewage sludge was superficially applied during the years 2000, 2001, 2002 and 2003 and doses were based on previous experimental results following the recommendations of the EPA (1994). No fertilization was applied later. An unlimed and unfertilized (UF) treatment was used as a control. Tree heights were measured using a vertex in the nine inner trees of each experimental plot in order to avoid the "border effect" in August 2008. Every year, pasture production was estimated before sward harvesting. No grazing was performed. Pasture production was estimated by collecting four random samples  $(1 \times 1 \text{ m}^2)$  per plot within the area occupied by 9 inner trees using battery-driven hand shears in spring and autumn 2008. Litterfall was estimated by hand separation of fractions of 100 g of biomass carried out to the laboratory in each harvest. Real pasture production was estimated by subtracting the area occupied by *Pinus radiata* basal tree crown canopy per hectare. In January 2008, a low pruning of trees was carried outto favour pasture production. SAS statistical software package (SAS, 2001) was used for all analyses.

#### **Results and discussion**

ANOVA results showed that the real pasture production was significantly influenced by dose (P < 0.05) and date (P < 0.01) of sewage sludge application.



Figure 1. Litterfall-Real pasture production relationship and Litterfall-Tree height relationship obtained in study plots in 2008. Where UF – unfertilized treatment, L – low dose (50 kg N total ha<sup>-1</sup>); H – high dose (100 kg N total ha<sup>-1</sup>); 1, 2, 3 – different dates of application of sewage sludge in February, March and April 2000–2004, respectively

Real pasture production was influenced by tree growth and litterfall biomass obtained in different study plots (Peri *et al.*, 2007) (Figure 1). The amount of real pasture production was smaller in those treatments that had increased tree height and litterfall biomass production in soil (LIME H1 and UNLIMED H3), and it was larger in those treatments



Figure 2. Real pasture production obtained in study plots in 2008. Where UF – unfertilized treatment, L – low dose (50 kg N total ha<sup>-1</sup>); H – high dose (100 kg N total ha<sup>-1</sup>); 1, 2, 3 – different dates of application of sewage sludge in February, March and April 2000–2004, respectively. Different letters indicate significant differences among treatments

with smaller litterfall biomass in the soil (LIME L2), due to litterfall biomass limiting the pasture growth (Figure 2).

#### Conclusions

Real pasture production was influenced by tree growth and litterfall biomass, being lower in those treatments that increased tree variables.

#### References

Chang S.X. and Mead D.J. (2003) Growth of radiate pine (*Pinus radiata* D. Don) as influenced by understory species in a silvopastoral system in New Zealand. *Agroforestry Systems* 59, 43–51.

EPA (Environmental Protection Agency) (1994) Land application of sewage sludge. A guide for land appliers on the requirements of the federal standards for the use and disposal of sewage sludge, 40 CFR Part 503. Washington, DC: Environment Protection Agency.

Gordon A.M., Naresh R.P.F. and Thevathasan V. (2005) How much carbon can be stored in Canadian agroecosystems using a silvopastoral approach? In: Mosquera-Losada M.R., McAdam J. and Rigueiro-Rodríguez A. (eds). *Silvopastoralism and land sustainable management*, CAB International, Wallingford, UK, pp. 210–219. Mosquera-Losada M.R., Cuiña-Cotarelo R. and Rigueiro-Rodríguez A. (2011) Effect of understory vegetation management through liming and sewage sludge fertilisation on soil fertility and *Pinus radiata* D. Don growth after reforestation. *European Journal of Forest Research* 130, 997–1008.

Montero M.J., Moreno G. and Bertomeu M. (2008) Light distribution in scattered-trees open woodlands in Western Spain. *Agroforestry System* 73, 233–244.

Peri P.L., Lucas R.J. and Moot D.J. (2007) Dry matter production, morphology and nutritive value of *Dactylis glomerata* growing under different light regimes. *Agroforestry Systems* 70, 63–79.

Rigueiro-Rodríguez A., Fernández-Núñez E., González-Hernández P., Mcadam J.H. and Mosquera-Losada M.R. (2009) Agroforestry Systems in Europe: Productive, Ecological and Social Perspectives. In: Rigueiro Rodríguez A., McAdam J. and Mosquera-Losada M.R. (eds). *Agroforestry in Europe. Advances in Agroforestry*, Springer, Netherland, pp. 43–66.

SAS (2001) SAS/ Stat User's Guide: Statistics. SAS Institute Inc., Cary, NC, USA, 943 pp.

# Evaluation of selected cultivars of fine-leaved fescues in conditions of extensive lawn utilization

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## Abstract

The aim of this work was to evaluate selected cultivars of fine-leaved fescues in conditions of extensive lawn utilization. In the years 1999–2010, the quality of biological traits of 24 cultivars of fine-leaved fescues was analysed under extensive lawn utilization (no irrigation, fertilization at  $N_{60}P_{20}K_{40}$  kg ha<sup>-1</sup> per year and cutting regimes of 4 times per year) in a completely randomized-design experiment with three replications on 1 m<sup>2</sup> plots). Cluster analysis was performed according to many biological traits carried out in 10-years of utilization and two different groups of cultivars were distinguished. The cultivars of sheep's-fescue Mimi, Noni and Witra, and also of red fescue Bargreen, Barnica, Nimba and Barcrown, were evaluated as better under the conditions of extensive lawn utilization, in terms of the investigated traits – particularly turf compactness, dynamics of sward regrowth and root weight in the sod layer.

Keywords: cultivar, extensive lawn, fine-leaved fescue

# Introduction

Fine-leaved fescues, in particular red fescue and sheep's-fescue, are well adapted to vegetative growth in difficult site conditions: they tolerate water shortage, reduced pH values, increased levels of salinity and toxic aluminium in the substrate, as well as poor soil fertility and, consequently, do not require fertilisation and irrigation for their growth and development (Ruemmele *et al.*, 1995; Rognli *et al.*, 2010). Adaptation of these grass species to extensive lawn utilization, as well as their considerable turf-forming capabilities, predispose them for use in the establishment of green areas on the basis of low-input sustainable turf (Diesburg *et al.*, 1997). However, the above-mentioned properties are, first and foremost, specific biological traits of cultivars of these grasses. For that reason, appropriate choice of cultivars for low-input sustainable turf is essential (Bourgoin, 1997). The aim of investigations was to assess selected cultivars of fine-leaved fescues in conditions of extensive lawn utilisation.

# Materials and methods

A study was carried out during 1999–2010, at the Brody Experimental Station (52° 26 N, 16° 18'E) of the Poznan University of Life Sciences, to determine the evaluation of selected cultivars of fine leaved fescues in conditions of extensive lawn utilization (no irrigation, cutting – four time per year, fertilization – in spring at the beginning of the vegetation period at the rate of  $N_{30}P_{10}K_{20}$  kg ha<sup>-1</sup> and after second cut at the end of June  $N_{30}P_{10}K_{20}$  kg ha<sup>-1</sup>). The field experiment was established in a completely randomized design in three replications with an experimental unit being a plot of 1 m<sup>2</sup> and situated on sandy soil (pH<sub>KCI</sub> – 5.4, N<sub>t</sub> – 0.62%, P – 37.6 mg kg<sup>-1</sup>, K – 93.0 mg kg<sup>-1</sup>, Mg – 30.0 mg kg<sup>-1</sup>) in which 24 cultivars were analysed. The cultivars were classified to *Festuca rubra* L. *ssp. genuina* Hackel = *rubra* Gaudin (*Frr*), *ssp. trichophylla* (Ducros ex Gaudin) K.Richter = *ssp. litoralis* (G.Meyer) Auquier (*Frt*),
ssp. fallax (Thuill.) Nyman = ssp. commutata Gaudin (Frc), Festuca ovina L. ssp. vulgaris (Koch) Schinz & Keller (Fov), Festuca ovina L. ssp. duriuscula (L.) Koch (Fod) and Festuca filiformis Pourret (Ff). The annual mean temperature and annual precipitation in the investigation period ranged from 8.9 to 9.6°C and from 549.4 to 664.0 mm, respectively. In the vegetation season, short-term drought periods occurred. The evaluation of cultivars of fine-leaved fescues was carried out in each year on the basis of the following traits: content of chlorophyll a+b in leaf blades in spring, sward height (herbometer) in the first regrowth, dynamics of sward regrowth, the yearly weight of the sward, weight of aboveground part of turf, content of lignins and cellulose in dead shoots in above-ground part of turf, sward compactness (Weber frame) after last cutting, as well as the root weight in the sod layer on the basis of cylinder samples (8 cm depth, 6 cm diameter) after washing and drying at 106°C determined before the end of the vegetation period.

Bearing in mind the fact that all the examined cultivar traits were continuous random variables, they were grouped with the assistance of cluster analysis using Ward's agglomeration procedure (Ward, 1963). Ward's method was applied as it provides clusters with the smallest possible internal variances. In order to make the results of cultivar grouping independent of measurement units, standardised traits were used for analysis. Following the analysis of traits for a 10-year period of utilisation, a diagram was obtained containing Euclidean distances indicating groups of cultivars of the highest degree of association with respect to the examined biological traits.

## **Results and discussion**

In the course of the 10-year period of utilisation, the sward compactness kept declining in the majority of red fescue cultivars and remained at a high level in sheep's-fescue cultivars. Nimba (Frc) and Barcrown (Frt) as well as Mimi (Fod), Noni (Fov) and Witra (Fov) cultivars deserve positive evaluation. Fine-leaved fescues varied also with respect to their dynamics of sward regrowth. Fast regrowth rates, especially during the first regrowth, were recorded in the case of Areta, Atra, Reda and Adio cultivars, i.e. genotypes classified to Frr., whereas Barcrown (Frt) and Barnica (Frc), as well as Fov and Fod cultivars were found to be characterised by slow regrowth rates. Results of experiments carried out by Huykebroeck et al. (1999) demonstrated that growth rates of red fescue cultivars were associated with plants photosynthetic economy. Genotypes classified as Frr, characterised by slow regrowth, exhibited high levels of light compensation as well as low respiration during the dark phase. On the other hand, Frr cultivars growing faster were characterised by a reverse light economy characteristics. It was found that Frc cultivars (Lovisa, Bargreen) formed lower swards and, consequently, smaller yearly weight of the sward in contrast to Frr cultivars (Atra, Areta and Barma). In this way, results reported by Huykebroeck et al. (1999) were corroborated. Also Goliński and Xi (2000) reported more rapid growth as well as development of higher sward by Frr cultivars (e.g. Bargena) in comparison with Frc cultivars. In our own studies, fine-leaved fescue cultivars were also shown to vary with respect to the weight of the above-ground part of the turf, as well as in lignin and cellulose content of dead shoots in the above-ground part of turf, i.e. in elements which exert a decisive effect on thatch formation. It was further found that the highest root weight in the sod layer, from among the examined red fescue cultivars, was developed by Bargreen Frc, while the lowest was by Barma Frr. Extensive development of the root weight in the 0–10 cm topsoil layer by the Bargreen cultivar was also emphasised by Goliński and Xi (2000). According to the results of our investigations, Mimi (Fod) and Noni (Fov) cultivars were characterised by even higher root weight in the sod layer.



Figure 1. Dendrogram of fine-leaved fescues cultivars according to results from a 10-year period of extensive lawn utilization (Ward's method, standardized traits)

Analysing the dendrogram of the examined traits of fine-leaved fescue cultivars in the course of the 10-year period of extensive lawn utilisation (Figure 1), two groups of these genotypes can be distinguished. The first group comprised all sheep's-fescue cultivars, Bargreen (Frc), Barnica (Frc) and Nimba (Frc) cultivars as well as the Barcrown (Frt) cultivar. The remaining cultivars formed one group from the point of view of Euclidean distances of binding, despite their different taxonomic classification. It is also worth indicating a significant similarity of chewings fescues. The response of the Barcrown cultivar to extensive lawn utilisation was also interesting. Despite its development of short rhizomes, it exhibited a similarity to chewings and sheep's-fescues.

#### Conclusions

Following the performed cluster analysis of many biological and use traits of selected cultivars of fine-leaved fescues during the 10-year period of extensive lawn utilisation, two different groups were distinguished. The first included cultivars of sheep's-fescue as well as Bargreen, Barnica, Nimba and Barcrown red fescues which were characterised by better lawn quality, especially with regard to sward compactness, slow regrowth rates as well as higher root weight in the sod layer. The second group was made up of a large number of fine-leaved fescue cultivars whose evaluation in conditions of extensive lawn utilisation was less favourable.

#### References

Bourgoin B. (1997) Variability of fine-leaved fescues (*Festuca* spp.) grown at low nitrogen levels. *International Turfgrass Society Research Journal* 8, 611–620.

Diesburg K.L., Christians N.E., Moore R., Branham B., Danneberger T.K., Reicher Z.J., Voigt T., Minner D.D. and Newman R. (1997) Species for low-input sustainable turf in the U.S. upper Midwest. *Agronomy Journal* 89, 4, 690–694.

Goliński P. and Xi Q. (2000) Evaluation of turf quality of some selected cultivars of *Festuca rubra* in sowing year set against varying soil humidity. *Grassland Science in Poland* 3, 43–50.

Huylenbroeck van J.M., Lootens P. and Bockstaele van E. (1999) Photosynthetic characteristics of perennial ryegrass and red fescue turf-grass cultivars. *Grass and Forage Science* 54, 267–274.

Rognli O.A., Saha M.C., Bhamidimarri S. and Van der Heijden S. (2010) Fescues. In: Boller B. *et al.* (eds), *Fodder Crops and Amenity Grasses, Handbook of Plant Breeding 5*, Springer Science+Business Media, 261–292.

Ruemmele B.A., Brilman L.A. and Huff D.R. (1995) Fine fescue germplasm diversity and vulnerability. *Crop Science* 35, 313–316.

Ward J.H. (1963) Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association* 58, 236–244.

# Festulolium (*x Festulolium* Asch. & Graebn.) and hybrid ryegrass (*Lolium x boucheanum* Kunth.) seed yield formation at two N fertiliser rates in Latvian climatic conditions

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# Abstract

The objective of this research was to investigate seed yield of *Festulolium* and *Lolium x* boucheanum cultivars at two nitrogen (N) fertiliser rates under the agro-ecological conditions of Latvia. Field trials were established on sod – gleyic soil with two nitrogen fertilisation rates (90 and 120 kg ha<sup>-1</sup> N). Seed yield is a highly complex trait which is influenced by a number of factors. The factor 'cultivar' had the greatest influence in terms of differences among seed yields, and it was significant. The factor 'year' representing a total of meteorological factors gave significant differences in seed yields. The increase of N fertiliser rate provided a significant increase of the mean seed yields; nevertheless, the comparatively low influence of factors allows the conclusion that an N level of 120 kg ha<sup>-1</sup> is close to the optimal nitrogen fertiliser rate in hybrid ryegrass and festulolium swards, providing seed yields of about 600 to 1000 kg ha<sup>-1</sup>.

Keywords: Festulolium, Lolium x boucheanum, seed yield formative elements

# Introduction

New hybrid grasses have recently been introduced in Latvia. *Festuloliums* are known to be among the most prospective herbage species in the context of development of sustainable agriculture, combining high forage quality and resistance to climate stress in many Europe countries (Nesheim and Bronstad, 2000; Nekrošas and Sliesaravičus, 2002; Kohoutek *et al.*, 2004).

Genetic instability and sterility of the interspecific hybrids is a limiting factor for festulolium and hybrid ryegrass breeding with regard to the production of commercial cultivars (Thomas and Humphreys, 1991). Seed yield has to become an important objective of research programmes, because even top-yield cultivars are not competitive on the seed market if their seed yield is poor (Nösberger and Staszewski, 2002).

The seed productivity of grasslands depends mostly on the grass cultivars used, and each region needs varieties that combine specialized combinations of stress resistance that are more appropriate and more productive for local climate conditions. Seed yield is a highly complex trait which is influenced both by genetic and environmental factors. In order to obtain a good perennial grass seed yield it is important to meet the following preconditions: high enough temperature and appropriate moisture during the flowering and seed maturity stage, as well as the use of proper cultivars with high seed yielding capacity and providing adequate fertilisation (Bumane and Bughrara, 2003).

# Materials and methods

Field trials were conducted in Latvia on a calcareous sod – gleyic soil (*Luvic Epigleyic Phaeozem* (*Calcaric*) – *WRB 2006*), fine sandy loam (20 to 30 cm deep arable layer, soil  $pH_{KCI} - 7.2$ , high plant available phosphorus and good potassium content, humus content 31 g kg<sup>-1</sup>). Eight cultivars were investigated: *Festulolium braunii* cv. Perun (*L. multi-florum x F. pratensis*), cv. Punia (*L. multiflorum x F. pratensis*), *Festulolium loliaceum* 

cv. Saikava (*L. perenne x F. pratensis*), *Festulolium pabulare* cv. Lofa (*L. multiflorum x F. arundinacea*) *x LM*, cv. Felina and cv. Hykor (*L. multiflorum x F. arundinacea*) *x FA*, hybrid ryegrass cv. Tapirus and cv. Ligunda (*L. multiflorum x L. perenne*). The trials were sown in May 2002, 2003 and 2004 without a cover crop, in a complete block design with four replications, each with a recorded plot area of 8 m<sup>2</sup>. The seeding rate was 600 viable seeds m<sup>-2</sup>. The plots were fertilised as follows: 104 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, 150 kg ha<sup>-1</sup> K<sub>2</sub>O and two N fertiliser treatments (90 and 120 kg ha<sup>-1</sup> N, i.e. N 90 and N 120 respectively). The seed yields were obtained in the first production year. The experimental data were statistically analysed using analysis of variance.

#### **Results and discussion**

Three years mean values for seed yields showed that the highest yields of seeds were produced by loloid type festulolium cultivars cv. Lofa (1252 kg ha<sup>-1</sup>) and cv. Saikava (1049 kg ha<sup>-1</sup>), while hybrid ryegrass cv. Tapirus (686 kg ha<sup>-1</sup>) gave the lowest yields of seeds (Table 1). Although the seed yield (one trial year results) of the early hybrid ryegrass cv. Ligunda was the lowest compared to other cultivars, the mean seed yield (641 kg ha<sup>-1</sup>) was sufficiently high, however, despite the poor winter hardiness and low productive tiller number. It shows potential for possibly high seed yields under the climatic conditions of Western Europe; however, this cultivar is not suited to Latvian climatic conditions.

Increasing the amount of nitrogen fertiliser from 90 to 120 kg ha<sup>-1</sup> provided significant increase in seed yields for all trial cultivars. With the increase of seed yields under the influence of nitrogen fertiliser, differences between festulolium cultivars of both types are not stated. For the loloid-type festulolium cultivars Saikava, Perun, Punia and Lofa, the average seed-yield increase was 122 kg ha<sup>-1</sup> or 13%, but for festucoid-type festulolium cultivars Felina and Hykor it was 100 kg ha<sup>-1</sup> or 12%. For hybrid ryegrass cultivars, the average seed yield increase was 65 kg ha<sup>-1</sup> or 10%.

N roto ka ha-l	Cultivars										
N Tate, Kg Ha	Ligunda	Tapirus	Punia	Hykor	Perun	Felina	Saikava	Lofa			
N 90	606	656	690	775	792	909	1010	1196			
$S_{\overline{x}}$	33	35	33	37	25	49	87	70			
N 120	675	717	821	900	960	984	1088	1308			
$S_{\overline{X}}$	37	41	25	27	46	36	77	64			
Mean	641	686	755	838	876	947	1049	1252			
$S_{\overline{x}}$	26	27	24	26	31	31	57	48			

Table 1. Seed yield, kg ha<sup>-1</sup> (average of three sowing cycles)

Analysis of factor influence h, % (eta) on seed yield showed that influence of cultivar factor was greatest (h, % = 52.3) and it was significant (P < 0.05) (Table 2). Under the influence of meteorological conditions in a particular year, seed yields of grasses may fluctuate considerably. The influence of meteorological factors was estimated by comparing different trial years as a total of meteorological factors in a specific trial year. The factor year gave significant (P < 0.05) differences in seed yields, and its influence (h, % = 15.6) was comparatively high. Although the increase of nitrogen fertiliser rate provided a significant (P < 0.05) increase of the mean seed yields, the comparatively low (h, % = 4.6) influence of this factor allows the conclusion that N 120 kg ha<sup>-1</sup> is close to the optimal nitrogen fertiliser rate in hybrid ryegrass and festulolium swards used for seed production.

There was a significant effect (P < 0.05) of the interaction between variety and year on the seed yield. This effect (h, % = 16.9) was higher than the influence of climatic (meteorological conditions) factor.

Source of Variation	SS	df	MS	F	F <sub>0.05</sub> crit
Cultivar (C)	5200052	5	1040010	130.66	2.30
N fertiliser rate (N)	455625	1	455625	57.24	3.93
Year of grass trials (Y)	1553229	2	776615	97.57	3.08
Interaction $C \times N$	45833	5	9167	1.15	2.30
Interaction $C \times Y$	1678750	10	167875	21.09	1.92
Interaction N × Y	77188	2	38594	4.85	3.08
Interaction $C \times N \times Y$	78229	10	7823	0.98	1.92
Error	835747	105	7959		
Total	9942656	143			

Table 2. Analysis of variance for festulolium and hybrid ryegrass cultivars seed yields

## Conclusions

Seeds yields for festulolium and hybrid ryegrass were dependent on the cultivar to be used and nitrogen fertiliser rate. Seed yield was found to be strongly dependent on climatic condition in the particular year.

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# References

Bumane S. and Bughrara S.S. (2003) Optimization of mineral nutrition for perennial ryegrass seed production. *Proceedings of the Latvia University of Agriculture* 8, 70–73.

Kohoutek A., Odstrcilova V., Komarek P. and Nerusil P. (2004) Persistence and production ability of *Dac*tylis glomerata L., *Dactylis polygama* Horvat, *Festuca arundinacea* L. and genus hybrids in 1986–2003. *Grassland Science in Europe* 9, 422–424.

Nekrošas S. and Sliesaravičus A. (2002) Investigation of intergeneric ryegrass-fescue and inerspecific ryegrass hybrids developed in Lithuania. Žemdirbyste: Mokslo darbai 78, 158–163.

Nesheim L. and Bronstad I. (2000) Yield and winter hardiness of *Festulolium (Festuca x Lolium)* in Norway. *Grassland Science in Europe* 5, 238–240.

Nösberger J. and Staszewski Z. (2002) Overview of the changes in research on grasslands in Europe. *Grassland Science in Europe* 7, 17–27.

Thomas H. and Humphreys M.O. (1991) Progress and potential of interspecific hybrids of *Lolium* and *Festuca. Journal of Agricultural Science* 117, 1–8.

# Evaluation of the development and turf characteristics of lawn grass cultivars in the year of sowing

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# Abstract

The aim of the study was the evaluation of six cultivars of three lawn grass species – *Lolium perenne, Poa pratensis* and *Festuca rubra* in relation to the development and re-growth rate in the year of sowing. The experiment was a randomised complete block design in spring of 2010. Depending on the species, during vegetative growth, 3-7 cuts to 4 cm were made. The re-growth rate of the cultivars was evaluated on the basis of the aboveground parts of plants examined every 7-10 days. Traditional criteria were used for the evaluation of turf characteristics, such as slow re-growth, resistance to disease, general aesthetic appearance, fineness of leaf, colour and the density of sward and roots. In the preliminary period of plant development, all phases were occurring more rapidly in the cultivars of *L. perenne* compared with *P. pratensis* and *F. rubra*. Ssignificant differences among cultivars were recorded for the tested characteristics. At the end of vegetative growth period, the best sward and root density was with both *L. perenne* cultivars. Results showed that cultivars of *L. perenne* and *F. rubra* were similar for the examined characteristics.

Keywords: emergence, lawn cultivars, tillering, turf characteristics, re-growth, roots density

# Introduction

The general aesthetic appearance of the species of grasses is the main trait that determines the quality of lawns. The important feature influencing the value of the lawn cultivars is slow growth after cuts (Prończuk *et al.*, 1997; Rutkowska and Hempel, 1986). *Poa pratensis* L., *Lolium perenne* L. and *Festuca rubra* L. are species commonly used to establish different types of lawns. Therefore, understanding the differences in the rate of re-growth of cultivars of these lawn species may contribute significantly to the determination of the usefulness of a particular cultivar for this purpose and the frequency of mowing. The aim of the study was evaluation of 6 cultivars of 3 lawn grass species – *L. perenne*, *P. pratensis* and *F. rubra*, in relation to development and re-growth rate in the sowing year.

# Materials and methods

The study was established on 28 April 2010 at the Experimental Station SGGW in Warsaw on a mineral brown soil. The concentration of available phosphorus, potassium and magnesium were medium, and the soil pH<sub>KCl</sub> was 5.2. The experimental design was a randomized complete block with four replicates in plots each of 1 m<sup>2</sup>. The experiment was done according to the agro-technical recommendations concerning lawn establishment (Rutkowska and Hempel, 1986). The study material included six Polish and foreign cultivars of three lawn grass species: *P. pratensis* – cv. Alicja, Miracle, *F. rubra* – cv. Florentine, Barma, and *L. perenne* – cv. Stadion, Romeo. Mineral fertilizer (NPK) was applied at 160, 26 and 83 kg ha<sup>-1</sup> per year, respectively. During vegetation, 3–7 cuts to 4 cm were made depending on the species. The rate of re-growth of the cultivars was evaluated on the basis of the aboveground herbage examined every 7–10 days. The onset and full emergence of the

tillering phase (number of days from sowing date) were measured. The lawn properties of cultivars were assessed with the use of the 9-degree scale of visual quality; in this classification system 9° denotes the highest and 1° the lowest trait value (Domański, 1992). The following traits were assessed: general appearance, fineness of leaf, colour, density of sward (summer and autumn), resistance to disease and root density (autumn). The experimental data were statistically analysed by a one-factor analysis of variance and verified using the LSD test. The weather conditions during the vegetative period were favourable for grass growth (Figure 1). The mean temperature in the growing season (April-October) was 14.6°C and precipitation amounted 692 mm. The rainfall data were obtained from the weather station – Ursynów of SGGW Department of Meteorology and Climatology.



Figure 1. The meteorological conditions in the period of study (April – October 2010)

#### **Results and discussion**

All phases occurred more rapidly in the cultivars of *L. perenne* (Table 1). In the preliminary period of plant development there were no differences between cultivars of *P. pratensis* and *F. rubra*. Similar results were reported for some grass species by Harkot *et al.* (2006). At the beginning of the tillering phase, *P. pratensis* cv. Alicja and *F. rubra* cv. Florentine began tillering earlier than the Miracle and Barma cultivars.

,	1			
Species	Cultivar	Beginning of emergence	Full of emergence	Beginning of tillering
Poa pratensis	Alicja	12a	16a	51a
*	Miracle	12a	16a	54a
Festuca rubra	Florentine	12a	16a	30b
	Barma	12a	16a	34b
Lolium perenne	Stadion	7b	9b	28c
*	Romeo	7b	9b	26c

Table 1. Beginning and full of emergence and tillering phases (days after sowing date) of *Poa pratensis*, *Festuca rubra* and *Lolium perenne* 

Values in columns with the same letters (a,b,c) create homogenous groups.

Results revealed that the re-growth rate of all the tested lawn cultivars was differentiated during vegetation period and was formed in the  $1.1-22.4 \text{ mm day}^{-1}$  (Table 2). The highest rate of re-growth was observed for the *L. perenne* cultivars especially for Stadion (mean of growing season 12.6 mm day<sup>-1</sup>). The Alicja and Miracle cultivars were characterized by a slow re-growth rate (mean of growing season 6.5 and 7.0 mm day<sup>-1</sup>, respectively). Comparable results were obtained by Domański *et al.* (2011). The general appearance of tested cultivars ranged from 5.3° to 8.3° (Table 3). Cultivars Stadion and Romeo of *L. perenne* reached significantly better values compared with the others, while cv. Alicja of *P. pratensis* 

gave the worst value. We found that cultivars of *F. rubra* – Florentine and Barma had the best leaf-fineness (9°) in comparison with cultivars of *L. perenne* and *P. pratensis*, especially Alicja (5.5°). It was also observed that cv. Florentine was distinguished by a dark, lush green colour (9.0°). The results showed that both of the *L. perenne* cultivars had significantly better sward density values (7.7°) compared with the other cultivars tested. It was also observed that cv. Florentine and Barma of *F. rubra* had the best resistance to disease (*Puccinia striiformis*). At the end of the vegetation period of the sowing year it was noticed that both cultivars of *L. perenne* as well as cv. Alicja of *P. pratensis* created strong and inseparable roots in the uppermost soil layer (roots density 9.0° and 8.5°, respectively).

Table 2. Re-growth rates of lawn cultivars of *Poa pratensis* (Alicja, Miracle), *Festuca rubra* (Florentine, Barma) and *Lolium perenne* (Stadion, Romeo) during vegetation (mm day<sup>-1</sup>)

<b>X</b> 7 · 4		May	r		Ju	ne			Ju	ly			A	Augus	st		Ser	otem	ber	Octo-
variety		5								-										ber
	10	20	26	1	11	21	28	5	12	19	27	2	9	16	23	30	8	15	22	4
Alicja	2.4	1.8	4.0	5.1	4.9	5.9	2.7	3.8	5.5	6.1	5.9	12.4	6.5	10.2	14.5	13.3	12.6	1.1	5.4	6.0
Miracle	2.9	1.5	3.5	4.5	3.8	4.0	1.8	6.0	3.5	6.6	6.4	13.6	8.2	9.8	16.2	17.9	16.1	1.7	5.1	6.7
Floren- tine	3.7	4.0	7.9	10.1	9.8	9.0	7.6	10.8	12.7	2.0	7.9	19.3	9.2	16.0	17.7	22.3	19.5	1.3	3.0	6.2
Barma	2.7	4.7	8.2	11.7	11.4	15.0	8.2	15.1	19.5	3.6	9.9	23.5	13.5	8.4	17.3	18.9	16.9	2.1	5.5	7.6
Stadion	5.3	8.0	15.2	22.6	18.4	16.0	10.5	21.0	3.6	7.0	8.5	24.4	14.8	10.8	15.1	15.4	14.2	1.8	14.8	4.5
Romeo	7.1	8.0	10.8	16.7	16.4	15.0	10.8	22.7	3.9	7.4	8.6	21.0	12.7	12.3	8.5	8.3	8.7	1.3	11.4	3.6
LSD <sub>0.05</sub>	3.19	0.96	2.79	5.37	4.15	4.86	4.17	7.34	3.69	3.83	ns	7.11	3.90	ns	ns	8.26	6.41	ns	3.97	ns

Table 3. Evaluation of *Poa pratensis*, *Festuca rubra* and *Lolium perenne* cultivars according to the turf characteristics recorded on a 1° to 9° scale

Spagios	Cultiver	General	Leaf fine-	Colour	Sward	Resistance of	Poots donsity
species	Cultival	aspect	ness	Coloui	density	disease	Koots delisity
Poa pratensis	Alicja	5.3	5.5	6.3	4.4	6.0	8.5
	Miracle	6.3	7.0	7.8	4.9	7.8	7.8
Fosting willing	Florentine	6.8	9.0	9.0	4.8	9.0	6.5
r esiuca rubra	Barma	6.1	9.0	7.0	5.5	9.0	7.8
I alium nanama	Stadion	7.9	7.3	7.0	7.7	7.0	9.0
Louum perenne	Romeo	8.3	7.5	8.5	7.7	8.3	9.0
LSD ( $P \le 0.05$ )		0.88	1.34	1.13	0.68	1.29	1.46

#### Conclusions

The emergence and tillering process was shorter in the cultivars of *L. perenne* Stadion and Romeo. The slow growing cultivars in the sowing year were cv. Alicja and Miracle of *P. pratensis*. From among the examined turf cultivars the best general aesthetic appearance, density of roots and sward were found in *L. perenne* cultivars, especially Romeo while cultivars of *F. rubra* (Florentine and Barma) showed the best fineness of leaf, resistance to disease and good colour.

#### References

Domański P. (1992) System of research and assessment of lawn grasses in Poland. *Biuletyn IHAR* 183, 251–263 (in Polish with English summary).

Domański P. Andrzejewska J. and Iwicki R. (2011) Effect of utilization intensity on growth of lawn cultivars of Kentucky bluegrass (*Poa pratensis* L.). *Acta Scientiarum Polonorum*, *Agricultura* 10(3), 15–26. Harkot W., Czarnecki Z. and Powroźnik M. (2006) Emergence and installment of selected lawn grass varieties at different sowing dates. *Zeszyty Naukowe Uniwersytety Przyrodniczego we Wrocławiu* 545, 111–120. (in Polish with English summary).

Prończuk S., Prończuk M. and Żyłka D. (1997) Methods of synthetic evaluation of turfgrasses. *Zeszyty Problemowe Postępów Nauk Rolniczych*, 451, 125–133. (in Polish with English summary). Rutkowska B. and Hempel A. (1986) *Lawns*. PWRiL, Warszawa, 256 pp. (in Polish).

# Detection of virus infection in fungal tissue using visible and near infrared spectroscopy

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# Abstract

Infections with *Neotyphodium* and *Epichloë* endophytes are common in grasses and, in turn, virus infection is common in these endophytic genera. The objective of this study was to test the use of visible and near-infrared (Vis-NIR) spectroscopy as a tool to diagnose the presence of viral double-stranded RNA (dsRNA) in strains of the fungal endophyte *Epichloë festucae* isolated from *Festuca rubra* plants. Vis-NIR spectroscopy has several advantages when compared to the conventional methods, including environmental friendliness, as no chemical waste is produced. Ground, freeze-dried mycelium samples from virus-infected and virus-free *E. festucae* isolates were scanned in reflectance mode at 2 nm intervals (range of 400–2498 nm). A total of 124 mean spectra were recorded. Calibration models were developed using partial least-squares discriminant analysis (PLS1-DA). Applying our best regression model constructed with two sampling years, and using standard normal variate (SNV) combined with first derivative transformation to new validating data set (42 samples), we obtained a correct classification of 75% of the uninfected isolates, and up to 86% of the infected isolates. To conclude, the application of Vis-NIR technology may be considered as a potentially useful technique for the detection of viral infections in grass endophytes.

Keywords: near-infrared spectroscopy, virus infection, Epichloë festucae

# Introduction

Wild populations of *Festuca rubra* are often infected by the endophytic fungus *Epichloë festucae*. In a previous paper, a field study was done to examine the infection frequency of *E. festucae* in populations of *F. rubra* and to estimate the incidence of virus infection in *E. festucae* (Petisco *et al.*, 2010). Two viruses with genomes of 5.2 kbp (EfV1) and 3.2 kbp (EfV2) were detected in *E. festucae*. EfV1 is a member of the genus *Victorivirus* (Fam. *Totiviridae*), and EfV2 is thought to be a *Mitovirus* (Romo *et al.*, 2007). Although several methods have been used for the diagnosis of viral infections, none of them is ideal in terms of cost-effectiveness and speed.

Visible and near infrared spectroscopy (400–2500 nm) is a fast, and accurate technique that does not require chemical reagents, being environmentally friendly. The absorption of molecules in the NIR region is due to combinations and overtones of vibration such as stretching and bending of hydrogen-bearing functional groups like –CH, –OH, and –NH (Osborne *et al.*, 1993). Typical applications of NIR spectroscopy include food, pharmaceutical, and agrochemical quality control, medical diagnostics, environment and petrochemical industry. However, until recently NIR spectroscopy had not been used in virology. The objective of this study was to develop models using spectral Vis-NIR measurements to discriminate between virus infected and virus free strains of the endophytic fungus *Epichloë festucae*.

# Materials and methods

Isolates of *E. festucae* were obtained from *F. rubra* plants collected at different locations in natural semiarid grasslands of the province of Salamanca, in Western Spain. The presence of the viruses EfV1 and EfV2 was diagnosed using 93 isolates of E. *festucae* from 2008 and 31 from 2006. The dsRNA was purified from 0.5–1 g of mycelium by CF-11 cellulose chromatography. The products of each dsRNA extraction procedure were resolved by electrophoresis in 1% agarose gels.

For the acquisition of spectra, five freeze-dried and ground cultures of each isolate were scanned on a NIRSystem 6500 scanning monochromator (FOSS NIRSystems, Silver Spring, USA). Spectra from 400 to 2500 nm were acquired at 2 nm wavelength intervals in reflectance mode. A total of 124 mean spectra were recorded as log 1/R, where R is the intensity of reflected light at each wavelength. Nine different types of spectral pre-treatments were tested in an effort to improve calibration accuracy. The models were developed using the Vis-NIR raw data and processed spectra with a combination of multiplicative scatter correction (MSC), standard normal variate (SNV), first derivative (1D) and second derivative (2D). A more detailed description of these data pretreatments can be found in Petisco *et al.* (2008). Calibrations generated for the classification of virus infected and virus free isolates were developed and evaluated with separated calibration and prediction sample sets. Principal component analysis (PCA) was made to detect outliers or any clustering of the data and then, partial least-squares discriminant analysis (PLS1-DA), onto a dummy variable, was the method used to generate calibrations for the classification. The accuracy of classifications models was assessed in the external validation set on the basis of the percentages of correct classification.

## **Results and discussion**

All samples were used in subsequent chemometric analyses because no unusual or outlying samples were detected in the PCA analysis. The first two PCs explained 99.8% of the total variation in the raw spectra. The PLS1-DA classification rates according to infection and the number of PLS factors (F) for the external validation sets using different mathematical treatments are shown in Table 1. The best results were achieved in samples from 2006; in this case, we obtained a percentage of correct classification of 100% in both classes of *E. festucae* isolates (infected and uninfected) when applied the data transformed to 2D or its combination with MSC or SNV. With respect to the results obtained with the 2008 samples, 13 out of 16 samples were correctly assigned to the infected class (81%) whereas 10 out of 15 correct assignments were made in the uninfected class (67%).

		200	6		2008	3		2006+2008			
Data mea traatmant	(n = 11)				(n = 3)	31)		(n = 42)			
Data pre-treatment	Б	Virus	No virus		Virus	No virus	Б	Virus	No virus		
	Г	(n = 6)	( <i>n</i> = 5)	Г	( <i>n</i> = 16)	( <i>n</i> = 15)	Г	(n = 22)	(n = 20)		
Raw	4	100	80	1	75	47	6	73	65		
MSC	3	100	80	4	81	60	5	77	70		
SNV	3	100	80	4	81	60	6	82	75		
1D	2	100	80	1	62	47	5	73	75		
MSC+1D	2	100	80	2	81	67	4	73	75		
SNV+1D	2	100	80	2	81	67	3	86	75		
2D	4	100	100	1	62	53	4	73	65		
MSC+2D	1	100	100	3	75	60	4	73	55		
SNV+2D	1	100	100	3	75	60	4	82	75		

n – number of isolates; F – number of PLS factors; MSC – multiplicative scatter correction; SNV – standard normal variate; 1D – first derivative; 2D – second derivative.

These results were obtained after transforming the raw spectra to MSC+1D and SNV+1D pretreatments. For the model including both samples of 2006 and 2008, the best pre-processing was SNV+1D. In this case 86% of the infected samples and 75% of the uninfected samples were correctly predicted (Table 1). The differences in classification between years could be due to the different ways used for grinding the fungal samples each year, since the particle size affects strongly to the absorbance of the spectra. Also, culture age was different for the 2006 and 2008 samples. In addition, the better separation of virus infected and virus free samples in 2006 could be due to the fact that among the 93 samples of 2008, infections by EfV2, as well as mixed infections by both viruses occurred. In contrast, all the virus infected samples from 2006 were infected by the two viruses, making their virus load higher. Therefore the best results of 2006 could also be related to a lower variability in samples. In virology, NIR spectroscopy has been used for the diagnosis of HIV-1 infection (Bahamani et al., 2009), for the discrimination between healthy and TMV infected tomato plants (Xu et al., 2006) or for the prediction of SMV in soybean plant samples (Jinendra et al., 2010). This study is the first to demonstrate that Vis-NIR spectroscopy is able to detect viral infections in a fungus E. festucae.

## Conclusions

The application of the PLS-DA algorithm to visible and near-infrared spectra of fungal isolates was found to be a promising method for the detection of virus infection. Although the results obtained in this study could be considered as preliminary, the technique may be useful for future studies including large number of samples infected with other viruses associated to plant pathogenic or commercially produced fungi.

#### Acknowledgements

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#### References

Bahamani M.K., Khosravi A., Miri R., Iwabu Y., Ikuta K. and Sakudo A. (2009) Spectroscopic characterization of human immunodeficiency virus type-1 infected plasma by principal component analysis and soft independent modelling of class analogy of visible and near-infrared spectra. *Molecular Medicine Reports* 2, 805–809.

Jinendra B., Tamaki K., Kuroki S., Vassileva M., Yoshida S. and Tsenkova R. (2010) Near infrared spectroscopy and aquaphotomics: novel approach for rapid in vivo diagnosis of virus infected soybean. *Biochemical and Biophysical Research Communications* 397, 685–690.

Osborne B.G., Fearn T. and Hindle P.T. (1993) Practical NIR spectroscopy with applications in food and beverage analysis, Longman Group, UK, 200 pp.

Petisco C., Downey G., Murray I., Zabalgogeazcoa I., Garcia-Criado B. and Garcia-Ciudad A. (2008) Direct classification of related species of fungal endophytes (*Epichloë* spp.) using visible and near-infrared spectroscopy and multivariate analysis. *FEMS Microbiology Letters* 284, 135–141.

Petisco C., Garcia-Ciudad A., Vazquez-de-Aldana B.R., Garcia-Criado B. and Zabalgogeazcoa I. (2010) Incidence of *Epichloë festucae* in *Festuca rubra* plants of natural grasslands and presence of double-stranded RNA fungal viruses. *Grassland Science in Europe* 15, 729–731.

Romo M., Leuchtmann A., Garcia B., Zabalgogeazcoa I. (2007) A totivirus infecting the mutualistic fungal endophyte *Epichloë festucae*. *Virus Research* 124, 38–43.

Xu H., Zhu S., Ying Y. and Jiang H. (2006) Application of multispectral reflectance for early detection of tomato disease. In: The Society of Photo-Optical Instrumentation Engineers (eds). *Proceedings of the Society of Photo-Optical Instrumentation Engineers*, Boston, pp. 63810R.1-63810R.8.

# Performance of three turfgrass mixtures in the pedoclimatic conditions of NE Romania

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# Abstract

Turf is composed of perennial grass species and cultivars mixed in different proportions to create various types of turf that meet certain requirements of utilisation or adaptation to climatic conditions such as excessive drought or strong shading. In our study we analysed three different turf mixtures under the influence of differentiated fertilization and the climatic conditions in the NE region of Romania. The first mixture (A1) was composed of *Festuca arundinacea* 80% + *Lolium perenne* 10% + *Poa pratensis* 10%. The second mixture (A2) consisted of three cultivars of *Lolium perenne* mixed in equal proportions. The third mixture (A3) was composed of *Festuca rubra* 60% + *Lolium perenne* 20% + *Festuca ovina duriusculla* 10% + *Poa pratensis* 10%. Three types of fertilizers were applied: ammonium nitrate, a complex fertilizer with nitrogen and phosphorus, and a commercial lawn fertilizer with macro and micronutrients. The experimental design was a split plot design with three replicates. Mixtures reacted positively to all three types of fertilizers. The best quality was observed for the A2 mixture, consisting of three cultivars of *Lolium perenne*.

Keywords: turfgrass, mixture, fertilization, Romania

# Introduction

Using a single grass species for the establishment of a lawn will produce a very uniform and aesthetic green cover but the possibility of this lawn to persist under years of natural or artificial stress is limited. Each individual species has qualities but also limitations (Kellner, 1974). The latest changes in global climate have set a new perspective in terms of water conservation so that turf management must shift from an intensive management towards an extensive one (Morris, 2006; Waltz and Carrow, 2008; Githinji, 2009). The species selection for turf mixtures should consider drought-resistant species that can produce a good quality lawn in conditions of limited irrigation (Aronson, 1987; DaCosta, 2006; Richardson *et al.*, 2008). Testing the species performances under non-irrigated conditions and especially testing new combinations of species for the creation of improved turf mixtures is very important. The aim of this study is to identify which is the best-adapted turfgrass mixture for the pedoclimatic conditions of NE Romania under non-irrigated management.

#### Materials and methods

The studied material consisted of three mixtures of cool season perennial grasses. The first mixture (A1) consisted of *Festuca arundinacea* 80% + *Lolium perenne* 10% + *Poa pratensis*10%. The second mixture (A2) consisted of three cultivars of *Lolium perenne* mixed in equal proportions. The third mixture (A3) consisted of *Festuca rubra* 60% + *Lolium perenne* 20% + *Festuca ovina duriusculla* 10% + *Poa pratensis* 10%. Three types of fertilizers were applied: ammonium nitrate, a complex fertilizer with nitrogen and phosphorus and a commercial lawn fertilizer with macro and micronutrients. All three fertilizers were applied at a nitrogen rate of 75 kg ha<sup>-1</sup>. The experimental design was a split plot design with three replicates. The main plot was the turfgrass mixture type with an area of 8 square meters

(2 m  $\times$  4 m), and the sub-plot area was 2 square meters (1 m  $\times$  2 m) which represented the fertilization treatment. The evaluation of quality was done using a visual rating scale recommended by NTEP (National Turfgrass Evaluation Program, USA). Ratings are given on a scale of 1–9, where 9 represents the best quality and the perfectly green lawn, and 1 represents the worst quality. The obtained data were interpreted statistically by analysis of variance and limit differences.

# **Results and discussion**

The mean annual temperature in the area is 9.6°C and the annual rainfall is 518 mm, with drought occuring in September. The mixture A2 recorded the best quality in June and July being noted with 7.6 and 7.2, but recorded a drop in quality in August and September (Table 1). The mixture A1 had a lower quality in the first two months but maintained a relatively constant overall quality throughout the season. The A3 mixture had the poorest quality in all four months.

	1			
Turfgrass mixture	June	July	August	September
A1	6.8 <sup>NS</sup>	6.1 <sup>NS</sup>	5.9 <sup>NS</sup>	5.3*
A2	7.6*	7.2 <sup>NS</sup>	5.8 <sup>NS</sup>	5.1*
A3	5.30	5.8 <sup>NS</sup>	$3.9^{\circ}$	$3.1^{\circ}$
Control (field average)	6.6 <sup>c</sup>	6.4 <sup>c</sup>	5.2 <sup>c</sup>	4.5 <sup>c</sup>
LSD 0.05	0.8	1.0	0.8	0.2

Table 1. Turfgrass mixture overall quality (1-worst; 9-best)

\*Positive significance; <sup>0</sup>Negative significance; <sup>NS</sup>Not significant; <sup>C</sup>Control.

Turf quality increased significantly with all three types of fertilizers. The best result was obtained at the fertilization complex with macro- and micronutrients (Table 2).

	÷			
Fertilization	June	July	August	September
Control $(N_0 P_0 K_0)$	5.3 <sup>c</sup>	5.6 <sup>c</sup>	5.0 <sup>c</sup>	4.0 <sup>c</sup>
N <sub>75</sub>	6.7*	6.6*	5.1 <sup>NS</sup>	4.3 <sup>NS</sup>
$N_{75}^{'5} P_{45}$	7.0*	6.5*	5.1 <sup>NS</sup>	4.7*
$N_{75} P_{45} K_{72} + Ca_{18} Mg_{15} S_{14} + ME$ (Fe,	7.2*	6.6*	5.5 <sup>NS</sup>	4.9*
Zn, B, Mn, Mo)				
LSD 0.05	0.5	0.4	0.6	0.5

Table 2. The influence of fertilization on overall turfgrass quality (1-worst; 9-best)

\*Positive significance; <sup>0</sup>Negative significance; <sup>NS</sup>Not significant; <sup>C</sup>Control; ME microelements.

The best density was observed in mixture A2 rated with 7.8, followed by mixture A3 rated with 6.7, but the differences were not significant (NS). The highest percentage of ground cover was observed for the mixture A2, at 91%, and the lowest was for mixture A3, being only 70% (Table 3).

Table 3. Turfgrass mixture density (1-lowest; 9-highest) and ground cover (%) in the summer

Turfgrass mixture	Density	Ground cover %
A1	6.5 <sup>NS</sup>	87 <sup>NS</sup>
A2	7.8 <sup>NS</sup>	91 <sup>NS</sup>
A3	6.7 <sup>NS</sup>	$70^{0}$
Control (field average)	7.0 <sup>c</sup>	83 <sup>c</sup>
LSD 0.05	0.8	10

\*Positive significance; <sup>0</sup>Negative significance; <sup>NS</sup>Not significant; <sup>C</sup>Control.

Turfgrass density increased significantly from 5.9 to 7.6 when we used a complex fertilizer with macro and micronutrients. The ground cover increased from 73% to 88% at the fertilization with  $N_{75}P_{45}$  (Table 4).

Fertilization	Density	Ground cover %
Control $(N_0 P_0 K_0)$	5.9 <sup>c</sup>	73 <sup>c</sup>
N <sub>75</sub>	7.1*	84*
$N_{75}^{'5} P_{45}$	7.3*	88*
$N_{75}^{''} P_{45}^{''} K_{72}^{'} + Ca_{18}^{'} Mg_{15}^{'} S_{14}^{'} + ME$ (Fe, Zn, B, Mn, Mo)	7.6*	85*
LSD 0.05	0.6	4

Table 4. The influence of fertilization on turfgrass density (1-lowest; 9-highest) and ground cover (%)

\*Positive significance; <sup>0</sup>Negative significance; <sup>NS</sup>Not significant; <sup>C</sup>Control.

The highest content of phosphorus was observed for the mixture A1 (0.47% phosphorus) in the case of the complex fertilization with macro- and micronutrients. In general, phosphorus content decreased at fertilization with nitrogen ( $N_{75}$ ) compared to control plot ( $N_0 P_0 K_0$ ).

Table 5. The influence of fertilization and turfgrass mixture on phosphorus content (%) in plant tissue

Turfgrass mixture	$Control (N_0 P_0 K_0)$	N75	N75 P45	$\frac{N_{75} P_{45} K_{72} + Ca_{18} Mg_{15} S_{14} +}{ME (Fe, Zn, B, Mn, Mo)}$
A1	0.38	0.32	0.45	0.47
A2	0.47	0.43	0.43	0.45
A3	0.39	0.36	0.38	0.38

# Conclusions

The best quality was recorded for the A2 mixture, consisting of three cultivars of *Lolium perenne*, in terms of overall quality. The second-best turfgrass mixture was the A1 mixture, which recorded significant differences in September. The mixture with a high content of *Festuca rubra* had a poor quality and negative significant differences in June, August and September. Phosphorus content of plants decreased under the fertilization with nitrogen ( $N_{75}$ ) and increased under fertilization with  $N_{75}P_{45}$  and the complex fertilizer with macro-and micronutrients.

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# References

Aronson L.J., Gold A.J. and Hull R.J. (1987) Cool-season turfgrass responses to drought stress. *Crop Science* 27, 1261–1266.

DaCosta M. and Bingru H. (2006) Minimum water requirements for creeping, colonial, and velvet bentgrasses under fairway conditions. *Crop Science* 46, 81–89.

Githinji L.J.M., Dane J.H. and Walker R.H. (2009) Water-use patterns of tall fescue and hybrid bluegrass cultivars subjected to ET-based irrigation scheduling. *Irrigation Science* 27, 5, 377–391.

Kellner E. (1974) Gazonul. Editura Ceres București.

Morris K.N. (2006) The National Turfgrass Research Initiative. USGA Turfgrass and Environmental Research Online 5(10), 1–10.

Richardson M.D., Karcher D.E., Hignight K. and Rush D. (2008) Drought tolerance and rooting capacity of Kentucky Bluegrass cultivars. *Crop Science* 48, 2429–2436.

Waltz F.C. and Carrow R.N. (2008). Applied turfgrass water-use efficiency/conservation: Agronomic practices and building cooperation between industry trade associations and regulatory authorities. Proceedings of the International Conference on Turfgrass Science and Management for Sports Fields. *Acta Horticulturae* 783, 239–245.

# Effects of spring nitrogen application on Italian ryegrass seed production and its competition with cleavers

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# Abstract

A field study in western Serbia evaluated during two seasons (from 2005 to 2007) the effect of nitrogen (N) fertilizer application on Italian ryegrass (*Lolium italicum* Lam.) grown for seed and its competitive interaction with cleavers (*Galium aparine* L.) in the year after establishment. Increasing rates of N fertilizer (control, N<sub>1</sub>-50, N<sub>2</sub>-100 and N<sub>3</sub>-150 kg ha<sup>-1</sup> N) and two ryegrass sowing rates (5 and 20 kg ha<sup>-1</sup>) were used. The increase of fertilizer N level improved competitive ability of ryegrass and affected the interspecific competitive interactions. The interaction between Italian ryegrass and cleavers was evaluated by measuring total nitrogen content (TNC) in the leaf during vegetation and seed yield at the end of vegetation period. The highest seed yield was obtained with 100 kg ha<sup>-1</sup> N in both experimental years as well as in ryegrass seed yield.

Keywords: cleavers, Italian ryegrass, nitrogen fertilizer, sowing rate, seed yield

# Introduction

Weed competition in grass-seed fields can reduce plant vigour and seed yield, making weed control a major concern in most grass-seed fields. Grass weeds are especially troublesome because, if left uncontrolled, they can contaminate the seed harvest with undesirable seeds. Italian ryegrass (IR), a short-term but valuable forage grass, is appropriate as a model plant for spring nitrogen (N) absorption (Simić et al., 2009). The N content is the greatest individual nutrition factor affecting the growth and development of IR (Griffith and Chastain, 1997). Competition for N between seed crop and weeds is influenced by N amount and weed species (Nakova, 2008). Cleavers is one of the commonest broad-leaved weeds of graminaceous crops; it competes with the crop, delays harvest and contaminates harvested grain (Bain and Attridge, 1988). It is considered to be the most competitive weed in cereals in Serbia and causes large economic losses in these crops. Cereals are commonly the preceding crops of grass-seed crops. Nitrogen application can increase reproduction and growth of cleavers (Nakova, 2008). One method to increase the competitiveness of a crop is to increase the planting density (Seefeldt and Armstrong, 2000). The field study was conducted to determine the methods for managing the seed production of IR during the first production year. Questions of plant N-uptake dynamics and ryegrass competitive interaction with weeds are posed.

# Materials and methods

The study was conducted during 2005–2007, near Šabac, Serbia (44°47' N, 19°35' E, 80 m a.s.l.) which is located in a semi-humid region. A tri-factorial trial (2 sowing rates  $\times$  4 fertilization rates  $\times$  2 crops – ryegrass monoculture and mixture ryegrass – cleavers) with three replications was set up under a RCB design with 10 m<sup>2</sup> plots, in 2005–2006 and 2006/2007. Italian ryegrass (cv. Tetraflorum) was sown in October during both years

at a sowing rate (SR) of 5 kg ha<sup>-1</sup> and 20 kg ha<sup>-1</sup> at 20 cm inter-row spacing. The fertilizer treatments (calcium ammonium nitrate, 27% N) were applied to the plots in spring of each year (N<sub>1</sub> – 0 kg ha<sup>-1</sup>; N<sub>2</sub> – 50 kg ha<sup>-1</sup>; N<sub>3</sub> – 100 kg ha<sup>-1</sup> and N<sub>4</sub> – 150 kg ha<sup>-1</sup>). Cleavers was not sown as there were sufficient natural reserves of seeds in the soil. All other weeds were removed by hand during the crop cycle. Total nitrogen content (TNC) was measured in leaves by the Kjeldahl method (Musinger and McKinney, 1982) using 30 randomly collected plants of Italian ryegrass and cleavers on each plot, at four times during the growing season of ryegrass (V1 – tillering; V2 – stem extension; V3 – flagleaf, and V4 – earing). TNC in plant material was used as a parameter of competitive interaction between the cultivated and weed species. Data were analysed by parametric tests (ANOVA and LSD test), and with Statistica 8.0 software packages. The main characteristics of the soil (depth: 0–30 cm) were as following: soil texture – loam; pH<sub>KCl</sub> – 5.25; K – 12.5 mg kg<sup>-1</sup> and P – 1.31 mg kg<sup>-1</sup>.

#### **Results and discussion**

TNC analysis, evaluating all data obtained during vegetation growth during two years, showed that N content is higher in IR than in cleavers (2.58% vs. 2.41%, on average, in 2006, and 2.45% vs. 2.40% in 2007). With a full N supply in young plants, ryegrass had a higher growth rate, but the differences in N content between IR and cleavers were diminished as the growing season progressed (Figure 1). There was an increased amount of N at the last measurement in 2006, both in IR and cleavers, which can be explained by late spring precipitation, which favoured secondary tillering during earing of IR. These ryegrass vegetative tillers, as well as cleavers secondary growth. enhanced the average N content. Young plants and increased N mineralization induced abundant N accumulation. A similar situation occurred in 2007, after cleavers regrowth from seed: average N content increased compared to the period before, while IR reversed from full flag leaf emergence leading to linear decreasing of TNC. The higher N rates improved the competitive ability of IR and affected the interspecific competitive interactions, similar to the competitive superiority of wheat against cleavers (Nakova, 2008). Similarly, it confirms that initial size differences between competing species had a greater influence on growth than plant density (Klem and Vanova, 2000). The fast growing rate of ryegrass plants at the onset of competition is a reason for their greater competitiveness. The effect of N supply on the ryegrass seed yield was quite evident during two years: the highest seed yield was obtained with 100 kg ha<sup>-1</sup> N (915 kg ha<sup>-1</sup> and 782 kg ha<sup>-1</sup> in 2006 and 2007, respectively). Nitrogen supply of 150 kg ha<sup>-1</sup> was inadequate and it reduced seed yield. A favourable effect of increasing ryegrass sowing rate was to enhance its competitiveness towards cleavers (934 and 813 kg  $ha^{-1}$  in 2006 and 2007, respectively), but it cannot justify the four-fold higher rates of IR. Ryegrass seed yield was higher in monoculture



Figure 1. Changes in pattern of Italian ryegrass (IR) and cleavers nitrogen content TNC (%) at four growing stages in 2006 and 2007 (means  $\pm$  standard error of the mean)

by 3.5% in 2006 and 13.3% in 2007, indicating that cleavers decreases grass seed yield. It is evident (Figure 2) that sowing rate and various application levels of nitrogen affected ryegrass seed yield in both years. Ryegrass seed yield loss due to the presence of weed plants was non-significant, suggesting that suppressive effects of cleavers might have been negated by ryegrass and that most of its yield components remained unaffected. Maximum seed yield of 752 kg ha<sup>-1</sup> (SR 5 kg ha<sup>-1</sup>) and 934 kg ha<sup>-1</sup> (SR 20 kg ha<sup>-1</sup>) was obtained in cleavers-free plots in year 2006. There is a potential for reducing herbicide use in IR seed production under cleavers infestation.



Figure 2. Italian ryegrass seed yield (kg ha<sup>-1</sup>) in competition with cleavers. IR sown at two sowing rates under four rates of nitrogen supply in 2006 and 2007; IR – Italian ryegrass (pure), IR+C-mixture of Italian ryegrass and cleavers

# Conclusions

Despite the high variability of the data, which can be attributed mainly to the management of the studied sites interacting with uncontrolled environmental sources of variation, it was possible to demonstrate that spring nitrogen application and higher seeding rates of Italian ryegrass may be effective in reducing the impact of weeds. This could be critical for the production and quality of this valuable grass seed under weed-prevalent conditions. The results indicate that in order to maximize IR seed productivity in the first production year, a higher seeding rate is preferable (20 kg ha<sup>-1</sup>). Also, the results support the use of a relatively high fertilizer N rate for IR seed production to reduce the impact of weeds.

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# References

Bain A.B. and Attridge T.H. (1988) A shade-light mediated responses in field and hedgerow populations of *Galium aparine* L. *Journal of Experimental Botany* 39, 1759–1764.

Griffith S.M. and Chastain T.G. (1997) Physiology and growth of ryegrass. In: Rouquette Jr. F.M., and Nelson L.R. (eds). *Ecology, Production, and Management of Lolium for Forage in the USA*, CSSA Special Publication Number 24, Madison WI. 15–28.

Klem K. and Vanova M. (2000) Asymmetrische Konkurrenz zwischen Weizen und Unkrautarten – ein Schritt zum Präzisieren des Kulturpflanzen-Unkraut-Interaktionsmodells. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 17, 113–119.

Musinger R.A. and McKinney R. (1982) Modern Kjeldahl System. American Laboratory 14, 76-79.

Nakova R. (2008) Reproduction of cleavers (*Galium aparine* L.) under different densities and nitrogen rates. *Acta biologica iugoslavica* – serija G: *Acta Herbologica* 17, 167–172.

Seefeldt S.S. and Armstrong M.L. (2000) Impact of perennial ryegrass seeding densities on weed emergence, growth and development. *New Zealand Plant Protection* 53, 38–43.

Simić A., Vučković S., Kresović M., Vrbničanin S. and Božić D. (2009) Changes of crude protein content in Italian ryegrass influenced by spring N application. *Biotechnology in Animal Husbandry* 25, 1171–1179.

# Studies on selected elements of seed propagation of minor grass species

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# Abstract

A wide range of seed material from different grass species is necessary to keep high quality grasslands and to create buffer zones between arable lands and forest, and also to re-cultivate waste or fallow land. Therefore, the aim of our research was to describe elements of seed propagation for some minor grass species. On the basis of field experiments, different spacing and seed quantities were investigated for *Beckmannia eruciformis*, *Cynosurus cristatus* and *Elytrigia elongata* aiming at an optimal seed production. Satisfactory seed yields were obtained even at a reduced (50% to 75%) amount of seed quantity, as compared to theoretical (or normal) values, calculated on the basis of number of plants per area unit.

Keywords: Beckmannia, Cynosurus, Elytrigia elongata, seed yields

## Introduction

Recent changes in agriculture, especially intensification and specialization, have led to a reduction of species and varieties used. Many grass species become less profitable and finally are no longer part of any breeding and seed propagation programmes. Grasses play an important role in broadening biodiversity and CO<sub>2</sub> sequestration, as well as pasture and forage functions. Multiplication of sufficient amount of seeds of different so-called minor grasses and their use for regeneration of native, species-rich meadow swards could be used as a possible way of increasing biodiversity. The term 'minor grasses' refers to the degree of attention paid to these species by scientists, plant breeders and by the commercial sector, etc. (Zurek and Sevcikova, 2010). Species as Beckmannia eruciformis, Briza media, Trisetum flavescens, Pucinellia distans or Cynosurus cristatus are no longer part of any breeding programmes, and there is lack of available seed material (Krzymuski *et al.*, 2003). Minor grasses are added as important components to site-specific, species-rich seed mixtures to increase plant species diversity of newly established grassland. In the past decades the importance of minor grasses has increased as a response to the public interest in restoration of disturbed landscapes by 'near-natural' methods of re-vegetation. Since 2008, in the Plant Breeding and Acclimatization Institute, National Research Institute, with financial support from Ministry of Agriculture and Rural Development, a scientific programme has been launched to improve seed propagation of minor grass species for green areas and grasslands. The aim of the current research was to test different recommendations for seed production of three selected grass species: Beckmannia eruciformis, Cynosurus cristatus and Elytrigia elongata.

#### Materials and methods

Seed material was kindly provided by the Polish Genebank, PBAI – NRI. A field trial was established in 2009 in Radzików, Poland. *Elytrigia* and *Cynosurs* originated from collections from Ukraine, while *Beckmannia* originated from a Polish collection. The experimental design included three replicates with two treatments: three levels of seed quantities and two different row distances (for details see Table 1). Each single plot of each species per treatment and per replication was 2 m<sup>2</sup>. Seed quantities were established at three levels: normal, ca. 50% less than normal, and ca. 75% less than normal. 'Normal' quantity was calculated for 75 plants for *Elytrigia elongata*, 500 plants for *Beckmannia eruciformis* and 1000 plants for *Cynosurus cristatus* per 1 m<sup>2</sup> of plantation. These values were calculated after Martyniak and Martyniak (2002) and Martyniak (2005). Fertilization was applied in spring (60 kg N ha<sup>-1</sup>) and in autumn (80 kg of P and K ha<sup>-1</sup>). The following traits were measured in 2010 and 2011: overwintering (OW, 1–9 scale, where 1 is dead plants), plant height at flowering time (PH, in cm), number of seed heads per m<sup>-2</sup> (SH), lodging (LD, on a 1–9 scale, where 1 means plants lying flat on the ground), rust susceptibility (RUST, 1–9, where 1 means plants are completely dead) and seed yield (SY, t ha<sup>-1</sup>). Seed harvest was performed manually at the full ripening phase for each species.

## **Results and discussion**

The investigated species showed significant differences in all measured traits (Table 1).

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Genus,	Sowing	Row		Traits r	neasured	and o	bserved <sup>1</sup>	
species	quantity (kg ha-1)	spacing (cm)	OW	PH	SH	LD	RUST	SY
Elytrigia	15.0 (normal)		7.0	180.0	426.7	5.3	9.0	0.87
elongata	7.5 (50% less)	25	6.7	181.7	492.0	5.3	9.0	0.92
-	4.0 (74% less)		6.0	190.0	434.7	6.0	9.0	0.74
	15.0 (normal)		6.0	180.0	586.7	5.7	9.0	0.76
	7.5 (50% less)	50	5.3	186.7	714.7	5.7	9.0	0.62
	4.0 (74% less)		4.7	188.3	736.0	6.3	9.0	0.63
Effect of:	years		ns	***	ns	***	***	***
	row spacing		ns	ns	ns	ns	ns	ns
	sowing quantity		ns	ns	ns	ns	ns	ns
Beckmannia	16.0 (normal)		3.3	106.7	1266.7	6.7	6.3	1.52
eruciformis	8.3 (47% less)	20	3.0	105.0	1179.3	7.0	7.0	1.66
	4.1 (74% less)		3.3	110.0	1074.3	7.7	7.0	1.57
	16.0 (normal)		4.0	105.0	1025.3	6.7	6.7	1.68
	8.3 (47% less)	30	3.7	106.7	1148.0	7.0	6.7	1.74
	4.1 (74% less)		3.3	106.7	1182.0	8.0	6.7	1.65
Effect of:	years		***	***	***	***	ns	***
	row spacing		ns	ns	ns	ns	ns	ns
	sowing quantity		ns	ns	ns	***	ns	ns
Cynosurus	10.0 (normal)		5.0	88.3	1536.0	7.0	4.0	1.02
cristatus	5.0 (50% less)	15	5.3	88.3	2040.0	7.0	4.3	0.96
	2.4 (76% less)		5.7	88.3	2008.0	8.0	4.7	0.90
	10.0 (normal)		5.7	91.7	1580.0	7.0	5.0	0.95
	5.0 (50% less)	25	5.7	88.3	1736.0	7.7	4.7	1.02
	2.4 (76% less)		6.0	91.7	1645.3	8.0	4.7	0.89
Effect of:	years		***	ns	***	***	ns	***
	row spacing		ns	ns	ns	ns	ns	***
	sowing quantity		ns	ns	ns	***	ns	ns
Differences between:								
years			ns	ns	***	***	ns	***
species			***	***	***	***	***	***
seed quantity			ns	ns	ns	***	ns	ns
row spacing			ns	ns	ns	ns	ns	ns

Table 1. Results of measurements and observation (means from 2010–2011), significance of effects of major sources of variation (only direct effects were shown)

<sup>1</sup> trait symbols refer to descriptions given in 'M&M', \*\*, \*\*\* – significance of difference at  $\alpha$  0.05 and 0.001.

Significant effect of years was noted for the majority of traits, including seed yield. However, in the case of the treatments examined, significant differences between the different sowing quantities were detected only for lodging. When species were sown at lower quantities (2.4, 4.0 and 4.1 kg ha<sup>-1</sup> for *Cynosurus, Elytrigia* and *Beckmannia*, respectively) lodging was stronger than when sown at higher quantities. The highest seed yields were obtained for *Beckmannia eruciformis* (species average - 1.64 t ha<sup>-1</sup>). It has to be mentioned that, after three or four years of satisfactory seed yields, *Beckmannia* usually degrades drastically and it is necessary to renew the plantation (Martyniak, personal communication). For *Cynosurus cristatus* the period for seed production is even shorter (probably due to serious rust infection), whereas *Elytrigia* plantation can be used much longer for seed propagation (5–7 years). What is of major practical importance is that none of the investigated treatments affected seed yield (Table 2). Therefore, it is possible to obtain satisfactory yields even at a reduced (50% to 75%) amount of seed quantity.

Seed yields of the above-mentioned grass species were not estimated frequently. Duke (1983) reported that *Elytrigia* cv. Orbit, cultivated in USA, yielded between 0.38 and 0.72 t ha<sup>-1</sup> which was similar to our results. According to breeder's information about the cv. Roznovska of *Cynosurus*, seed yield may range between 0.5–1.0 t ha<sup>-1</sup>; however, this was obtained at a seed sowing rate of 18–20 kg ha<sup>-1</sup> (OSEVA PRO, 2011). It is therefore reasonable to reduce the seed quantity according to our data. *Beckmannia* is currently rare in Poland and therefore information about seed yields is not available. Despite two cultivars having been developed and registered in Poland before the Second World War, nothing further has been done with seed production of *Beckmannia* (Krzymuski *et al.*, 2003).

## Conclusions

Seed production of the three mentioned minor grass species is currently running at PBAI-NRI, following the recommendations gained from the introduced research activity. Further intensive research is required for introduction of seed material into different grass areas.

# References

Duke J.A. (1983) Handbook of Energy Crops. http://www.hort.purdue.edu/newcrop/duke\_energy/refa-f.html Krzymuski J., Arseniuk E., Martyniak J. and Oleksiak T. (2003) *The history of breeding and seed production on Polish lands in XX Century*. Agricultural Crops. Pro-Druk Poznań, pp. 420 (in Polish).

Martyniak D. (2005) The effect of sowing quantity on plant density and yields of turf varieties of red fescue (*Festuca rubra* L.) in seed production. *Biuletyn IHAR* 237/238, 259–267 (in Polish).

Martyniak J. and Martyniak D. (2002) The effect of sowing quantity on plant density and yields in seed production of *Lolium perenne. Łąkarstwo w Polsce* 5, 145–154 (in Polish).

OSEVA PRO (2011) Poháňka hřebenitá (*Cynosurus cristatus* L.) ROŽNOVSKÁ. http://www.oseva.cz/zubri/ Żurek G. and Sevcikova M. (2010) Minor Grasses. In: Boller B., Veronesi F., Posselt U. (ed.) *Handbook of Plant Breeding*, vol. 5. Fodder Crops and Amenity Grasses. Springer Science +Business Media, New York, 381–394.

# Session 4

# Environmental and landscape resources

# Environmental impacts of grassland management at the plot and the farm scale

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## Abstract

Both positive and negative externalities are associated with grassland management, and huge differences in productivity and environmental impact prevail between species-rich extensive grasslands and input-intensive, frequently defoliated grass monocultures. Potentials for improving the eco-efficiency of grassland-based production systems through decisions regarding management intensity, nutrient management, grazing and forage conservation, and the use of temporary versus permanent grasslands are discussed at the plot and the farm scales. To do so, we carried out life cycle assessment studies and reviewed studies related to plant and animal diversity. Extensification is positive for farmland biodiversity but does not always imply an improvement of the product-related environmental performances with regard to air, soil and water or resource use. Besides management intensity, nutrient management emerges as the main lever to improve the environmental performances of grassland-based production. In contrast, forage conservation and the type of conservation procedure appear to be of minor importance, except for the effect on biodiversity. Compared to barn feeding, grazing showed strengths as well as weaknesses that varied by the scale at which the systems were compared. The effect of cultivating grass-clover temporary grasslands was mainly driven by the related modification in fertilization requirement.

Keywords: life cycle assessment, biodiversity, intensity, nutrients, grazing, conservation

# Introduction

Managed grasslands are acknowledged for their multifunctional role in producing food and delivering other important ecosystem services, such as biodiversity, soil conservation and the mitigation of pollution (MEA, 2005). Ruminant production systems are nevertheless also responsible for large emissions of greenhouse gasses (GHG) and other pollutants, and the delivery of services and the negative environmental impacts strongly depend on management (Kemp and Michalk, 2007). Optimization of grassland management faces the difficulty of trade-offs that exist between productivity and other ecosystem services, as well as between different impact categories (Pilgrim et al., 2010), and thus mitigation measures for one impact might aggravate another one. This highlights the importance of considering many environmental impacts when assessing the options for improved eco-efficiency of grassland-based production systems. Life Cycle Assessment (LCA) is proven to be a valuable method for the assessment of the environmental impacts of agricultural systems (Van der Werf and Petit, 2002). It analyses a product's entire life cycle (including the production, transport, and disposal of all related materials) and aims at evaluating all relevant environmental impacts of the activity under study. This paper evaluates management options regarding their influence on the product-related (per product unit produced) environmental impacts of grassland-based production systems. Specifically, we discuss options related to management intensity, nutrient management, grazing and forage conservation, and the use of temporary grasslands in separate sections, although interactions and trade-offs between these issues do exist.

# Material and methods

The discussion about the impacts of grassland-based agricultural production on air, soil and water is based on LCA studies using the SALCA method (Gaillard and Nemecek, 2009). At the plot scale, the production inventories describe model forage production systems of the Swiss lowland under favourable pedo-climatic conditions, while at the farm scale they originate from real farms. The system boundary was set at the farm gate. The plot scale method is described in Nemecek *et al.* (2011a; 2011b) and the farm scale method in Baumgartner *et al.* (2011). The impact categories encompass the demand for non-renewable energy resources (hereafter: energy demand), the global warming potential, the ozone formation potential, the losses of N and P to aquatic and terrestrial ecosystems (global eutrophication potential), the acidification potential, and the impacts of toxic pollutants on terrestrial and aquatic ecotoxicity potentials). We use the quantity of net energy for lactation (NEL) produced on the grassland as the reference (functional unit) for comparing management at the plot scale, thereby taking into account the nutritional quality of the forage. Discussion about the impacts on biodiversity is based on the SALCA-biodiversity method (Jeanneret *et al.*, 2006) and related literature.

## Influence of management intensity on air, soil and water

Intensive management is considered the main driver of pollution from agricultural land and diminishing land use intensity has been shown to reduce the negative impacts of agriculture per area unit (Nemecek et al., 2011b). But in the current context of increasing demand for milk and meat (FAO, 2002), improving the environmental performance per product unit is increasingly important. The intensive management scenario used here as baseline is based on good agricultural and environmental practices, with only 15.5 kg total N applied per tons of DM (Table 1). Decreasing management intensity from intensive management respecting good agricultural practices to lower intensity with some fertilization did not lessen the product-related environmental impacts of forage production (Figure 1a). Only when no fertilizer is applied (very low intensity, Figure 1a) can a clear reduction of the environmental burdens of the production be achieved. Forage from extensive meadows is nevertheless of low feeding value and is thus suited to a few feeding applications only. Applying high management intensity on two thirds and very low management intensity on one third of the surface would yield a similar NEL yield and a slightly higher average NEL concentration in the forage than applying a medium management intensity on the whole surface, with similar overall burdens on air, soil and water (Nemecek et al., 2011b). At the farm level, different intensity levels are associated with different farm types. Baumgartner et al. (2011) compared different Swiss farm types by means of LCA, using the functional units 'MJ of digestible energy for human nutrition' and 'ha per year'. Three farm types are discussed here: suckler cow farms, dairy farms and farms with pig fattening (more than 25% of the livestock units as pigs). Production intensity (per area) is lowest with suckler cow farms and highest with pig fattening farms, and the importance of grassland for forage production decreases from suckler cow farms through dairy farms to pig fattening farms. Suckler cow farms, which are based mainly on grassland, show the lowest environmental impacts per ha and year, followed by dairy farms, which in Switzerland largely use grasslands. The highest impacts per ha and year were found in pig fattening farms, which purchase large amounts of concentrates. On the contrary, when expressed per MJ of digestible energy produced, the impacts were highest for the suckler cow farms, due to their low productivity. Nguyen et al. (2010) studied beef from suckler cow and intensively reared dairy calves and found generally higher product-related impacts in the suckler cow system. In suckler cow systems, the entire impacts are allocated to beef production, whereas in combined dairy-beef systems, the burdens are shared between the milk and beef production. Haas *et al.* (2001) found lower impacts per ha for extensified and organic milk production systems, compared to intensive conventional systems. Per unit of milk produced, the global warming potential of the organic systems was nevertheless similar to that of the intensive conventional systems because the lower CO<sub>2</sub> and N<sub>2</sub>O emissions from the organic systems were compensated by higher CH<sub>4</sub> emissions. For milk production systems in New Zealand, Basset-Mens *et al.* (2009) found a lower negative impact of the low input system both per ha and per unit of produced milk.

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Management	Number of cuts	N fertilization	Gross yield	Energy in hay
intensity	per year	(kg total N ha <sup>-1</sup> yr <sup>-1</sup> )	$(t DM ha^{-1} yr^{-1})$	(MJ NEL kg <sup>-1</sup> DM)
High	5	209	13.5	5.8
Medium	4	142	11.0	5.2
Low	3	80	7.0	4.8
Very low	1	0	3.5	4.2

Table 1. Description of the management intensity scenarios at the plot scale

#### Influence of management intensity on biodiversity

SALCA-biodiversity estimates that biodiversity is favoured by low management intensity, while medium management intensity hardly improves biodiversity compared to intensive management. This is in agreement with field studies (e.g. Kleijn et al., 2009). A combination of plots managed at high and at very low intensity therefore appears more promising for maintaining biodiversity than management at medium intensity on the whole farmland area. Moreover, grasslands managed at different intensity shelter different plant communities with to some extent different species, and habitat heterogeneity is recognized as favourable for biodiversity at regional scale (e.g. Benton et al., 2003). For instance, of the 189 plant species found by Marini et al. (2008) in 45 meadows, 77 were found exclusively under extensive management, 4 exclusively under medium and 9 exclusively under high management intensity. But species richness of grassland plants is also positively influenced by the surface area of the habitat (Krauss et al., 2004). The size and the location of the plots should therefore be considered to maximize habitat area and connectivity within and between farms (Knop et al., 2011). The Swiss agri-environment scheme requests that farmers allocate a minimum of 7% of their agricultural area to ecological focus areas, in the mountains mostly extensive grasslands. Kampmann et al. (2012) showed that this scheme contributes to protection of plant diversity in mountain grasslands, despite the fact that the size of these species-rich plots is modest. In grazed plots, grazing intensity has a profound effect on invertebrates, although the response may differ depending on the vegetation type (Wallis de Vries et al., 2007; Jauregui et al., 2008). Intensive mowing activities or grazing modify the soil structure and humidity with negative impact on e.g. carabid diversity (Eyre et al., 1989), and extensively grazed pastures in general support a richer species assemblage of grasshoppers, bush crickets and snails than those that are intensively grazed (Batary et al., 2007; Boschi and Baur, 2007). Combined with local environmental factors and landscape configuration, high grazing intensity negatively influences the diversity of flowervisiting insects, like bees, butterflies and hoverflies (Sjodin et al., 2008).

#### Influence of nutrient management on air, soil and water

Mineral fertilizer and manure applications are associated with several potentially harmful emissions, like ammonia ( $NH_3$ ), nitrous oxide ( $N_2O$ ) and nitrate ( $NO_3$ ). Our LCA study at

the plot scale showed that the type of fertilizer considerably influences the impacts of forage production: mineral fertilization increased energy demand and aquatic and terrestrial ecotoxicity potential, but reduced eutrophication and acidification potentials when compared to organic fertilization (Figure 1b). The difference in global warming potential between organic and mineral fertilization remains small, because this potential is, with fertilized grasslands, determined to a large extent by emissions of N<sub>2</sub>O and CH<sub>4</sub>, rather than by CO<sub>2</sub> from the production of agricultural inputs. Both the eutrophication and the acidification potentials are reduced by mineral fertilization because it reduces the emission of NH<sub>2</sub>, which plays a predominant role for these two impacts and originates largely from slurry applications. The importance of NH<sub>3</sub> in the eutrophication potential also implies that the use of machinery reducing NH, losses during slurry application like the use of a band-spreader (Smith et al., 2000) allows for a clear reduction of this impact (Figure 1b). Reduction of NH<sub>3</sub> volatilization could lead to higher NO<sub>3</sub> leaching or N<sub>2</sub>O formation (Langevin et al., 2010). Splitting slurry application into many small quantities results in an increased NH<sub>3</sub> volatilization (Menzi et al., 1997), but NO, leaching could be reduced by this practice. Because of the predominant role of fertilization in the global warming potential, reducing N fertilization would significantly decrease this impact. Nyfeler et al. (2009) showed that grass-legume mixtures can produce yields reaching those of heavily fertilized grass crops with a N fertilization of only 50 kg ha<sup>-1</sup> yr<sup>-1</sup> thanks to symbiotic N fixation. This is not thought to increase  $N_0O$ emissions as direct N<sub>2</sub>O emissions from symbiotic fixation is considered negligible (IPCC, 2006). The scenario for grassland planted with a legume rich mixture and fertilized with 76 kg N ha-1 yr-1 compared with the scenario for grass-dominated grassland fertilized with 182 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Figure 1e) shows the response of SALCA to a partial replacement of N fertilization with symbiotic fixation. At the farm scale, using mineral N fertilizer on grasslands usually implies changes in manure allocation on the agricultural area and/or higher total N inputs. Del Prado et al. (2011) compared dairy farm scenarios using the SIMS<sub>DAIRY</sub> model, for which the number of cows, milk production and grass-maize-concentrate ratio in the diet was kept constant. One system imported mineral N, while the other used its manure for maize production and relied on N fixation for grass production. They calculated a 20% higher GHG emission for the system with mineral N than for the system without. The difference in GHG emission was therefore higher than in our study at the plot scale (+9%), because the emissions from manure utilization were similar in both systems.

#### Influence of nutrient management on biodiversity

Most plot experiments with fertilization on permanent grasslands show that increasing applications of mineral or organic fertilizer result in a drop in plant species richness and changes in botanical composition (e.g. Schellberg *et al.*, 1999). These trends, frequently observed in mid-term experiments, are confirmed both in long term experiments (Silvertown *et al.*, 2006), and in gradients of soil fertility on farmland (Isselstein *et al.*, 2005). The study of Kirkham *et al.* (2008) suggests that at similar levels of N, P and K applications, mineral fertilization is no more detrimental to species richness than farmyard manure. But as discussed by the authors, the amount of nutrient applied in the organic fertilizer treatments might in fact have been higher than in the inorganic treatments. Foster and Gross (1998) showed that indirect mechanisms may occur when litter accumulation due to increasing fertilization reduces species richness. Numerous authors have demonstrated that N influences botanical composition and species richness from an input of 30 kg N ha<sup>-1</sup> yr<sup>-1</sup> (e.g. Joyce, 2001; Mountford *et al.*, 1996; Plantureux, 1996). This threshold is lower if high patrimonial value species are considered, because of their high sensibility to soil nutrient

level (Grevilliot et al., 1998). Fewer studies focused on the influence of P and K on biodiversity, but most authors like Janssens et al. (1998) showed negative effects of soil P and K fertility on plant species diversity. Several works came to the conclusion that P is a key factor for plant diversity (e.g. Ceulemans et al., 2011). Phosphorus rich slurries, like pig slurries, might therefore be particularly detrimental to species diversity. Although most of the factors affecting animal wildlife on farmland act at the landscape level, several species groups strongly depending on local field conditions may be influenced by the application of mineral or organic fertilizers. These include organisms that mainly depend on soil conditions. Other effects of fertilization on animal wildlife are transmitted by indirect plant-animal interaction processes. Manure application generally supports a greater abundance of invertebrates that rely on non-degraded plant matter as food source, e.g. earthworms. In particular, it has been demonstrated that the application of farmyard manure strongly influences bacterial community structure, diversity, and biomass in soil (Hartman et al., 2006). Slurry, however, may differ in its effects. Ground beetles, rove beetles, ants and spiders were only marginally affected by the type of fertilization (Minarro et al., 2009), but it has been shown that mineral fertilizers can be detrimental to dung beetle diversity (Hutton and Giller, 2003).

#### Influence of grazing and forage conservation on air, soil and water

LCA analysis at the plot scale showed that grazing has contrasting effects compared to barn feeding of fresh grass, with clear trade-offs: while the energy demand and the ecotoxicity potentials are clearly reduced by grazing, the global warming potential increases because of increased N<sub>2</sub>O emissions (Figure 1d; Oenema et al., 1997). Management options to reduce  $N_0O$  emissions from grazed plots, like restricted grazing, are reviewed by Luo *et* al. (2010). The eutrophication potential was also estimated as slightly higher for the grazing scenario. Grazing can increase the risk of NO<sub>3</sub> leaching due to locally high N concentration in the urine spots. De Klein and Ledgard (2001) showed that reducing grazing could mitigate NO<sub>3</sub> leaching, but at the same time increase total N emissions. The results obtained at the plot level cannot be directly extrapolated to the farm level. The choice between barnfeeding and grazing implies a number of changes at the whole farm level, which may have strong implications for the efficiency of the systems. For instance, although grazing offers a significant potential for energy saving at the plot level, this savings potential does not always translate into a lower energy demand at the farm level. Nussbaum (2008) compared the energy efficiency of three grazing-based beef production systems with three barn-feeding based systems. The farms with barn-feeding had, on average, a slightly lower energy demand per kg of beef produced. Recently, a full grazing dairy system and a dairy system with mainly barn-feeding and minimal grazing have been compared in Switzerland (Sutter, 2011). The grazing system was characterized by a lower milk yield per cow but a lower use of concentrates, leading to clear advantages in the impact categories ecotoxicity (due to pesticide use for concentrate production) and deforestation (resulting from overseas import of concentrates). Furthermore grazing resulted in much less NH<sub>2</sub> emissions, since on the pastures the urine can infiltrate the soil before the urea is transformed to ammonium. As a consequence the grazing-based milk production had lower acidification and terrestrial eutrophication potentials. Due to higher feed consumption per kg of energy corrected milk (ECM) in the grazing system, higher  $CH_4$  emissions per kg of ECM were calculated, leading to less favourable global warming and ozone formation potentials. During the three experimental years, the grazing system improved from year to year, showing its potential to increase its efficiency. The relationship between grazing and CH<sub>4</sub> emissions is complex. In general, higher gross energy intake by the animals results in higher CH<sub>4</sub> emissions from



enteric fermentation and manure management (IPCC, 2006). One of the factors determining the energy intake is the digestibility of the feedstuffs. Gerber et al. (2010) concluded from a study on global dairy production that one of the most effective measures to reduce GHG emissions would be to increase the digestibility of the feed. The use of concentrates has been proposed as an effective way to reduce CH<sub>4</sub> emissions from enteric fermentation, but this has been questioned in recent years. The IPCC guidelines (IPCC, 2006) imply a lower emission factor only for systems with a very high concentrate ratio in the diet (at least 90%). O'Neill et al. (2011) found lower enteric CH, emissions in grazing dairy cows compared to cows fed with a total mixed ration. Flysjö et al. (2011) compared milk production in New Zealand and in Sweden by means of LCA. The production in New Zealand, mainly based on grazing, had a slightly lower global warming per kg of ECM than the Swedish system with high use of concentrates and higher milk yield per cow. However, there are also important climatic differences between these regions. Where continuous grazing is possible, good eco-efficiency can be expected with grazing: under climates with mild winters, no or little forage conservation and only minimal shelter for the animals are needed. This contributes to the favourable results of pasture-based milk and meat production in New Zealand compared to regions with a long winter feeding periods. Forage conservation requires numerous machinery operations but, compared to barn fed grass, conservation itself (Figure 1d) and the type of conservation (Figure 1c) only slightly modify the impacts of forage production (beside higher energy demand). Among the different types of conservation, only the use of barn hay drying systems considerably increases the energy demand and terrestrial ecotoxicity potential (Figure 1c). In grassland systems, pesticides only play a minor role in ecotoxicity impact categories. Emissions related to machinery operations are therefore largely responsible for these impacts.

#### Influence of grazing and forage conservation on biodiversity

The type of grassland use (grazing, cutting) and its features (intensity, dates, animal species, cutting height, spatial heterogeneity of grazing) influence species richness and botanical composition of grasslands. The influence of grazing on plant species richness can be positive, negative or neutral (Olff and Richie, 1998; Marriott et al., 2004). A general trend is thus difficult to draw because of interactions between management and the pedo-climatic conditions. In addition, some impacts on biodiversity depend on spatial scales: patch, community, grazing area, landscape. Cingolani et al. (2005) suggested that, for productive grazed grasslands, plant diversity shows a unimodal pattern, with low biodiversity in almost abandoned and intensively grazed pastures. The primary role of grazing animals for biodiversity is maintenance and enhancement of sward structural heterogeneity by selective defoliation, treading, nutrient cycling and propagule dispersal. At the same time grazing animals maintain high selection pressure on species (Rook and Tallowin, 2003). Díaz et al. (2007) analysed 197 studies conducted worldwide and conclude that grazing favours annual species, small plants, low plants (vs erected plants) and stoloniferous plants. While mineral fertilizers increase the homogeneity of soil fertility, nutrients deposited by grazing animals can provide heterogeneity beneficial to biodiversity. In most cases however, the mean plot increase in nutrient fertility offsets the heterogeneity, and plant diversity thus decreases (Reynolds et al., 2007). Low stocking rates can have positive impacts on bird habitats and ground dwelling mammals by influencing the vegetation structure (Fowler et al., 2004). Compared to grazed grasslands, extensive meadows often have a higher plant and arthropod species richness (Fischer and Wipf, 2002; Kruess and Tscharntke, 2002). The number of cuts per year and the earliness of the first cut during the growing season affect plant species richness (e.g. Zechmeister *et al.*, 2003). These two factors are usually interdependent. The date of first exploitation is a key element: an early cut excludes non-clonal species and a late cut results in dense biomass unsuitable for the settlement of new species (Smith *et al.*, 2002). The maximum specific diversity is generally obtained by quite late harvest dates: from mid-June to mid-July in European zones with oceanic or semicontinental climates (Critchley *et al.*, 2007). Britschgi *et al.* (2006) found that in alpine meadows, cutting earliness and frequency negatively affected the survival of the offspring of a typical meadow bird species. To maintain a sufficient breeding success for nesting birds it is crucial to establish a minimum time-window without cutting (Kragten and de Snoo, 2007). Whether the plant material is quickly exported after mowing (fresh grass, silage) or left on the field to dry (hay) is important for animal wildlife because in the later case, animals may escape after mowing. Mowing has a major negative impact on *e.g.* grasshoppers, the type of machine used being a major determinant of its impact (Humbert *et al.*, 2009). Furthermore, the subsequent treatment of the mown grass can have even more severe impacts on grasshopper populations than mowing itself (Humbert *et al.*, 2009).

#### Influence of temporary versus permanent grasslands on air, soil and water

The energy demand for soil preparation and sowing of temporary grassland is low compared to the total energy demand of the production, so for the same level of management intensity, the difference in energy demand between permanent and temporary grassland is small (Figure 1e). Soil preparation nevertheless triggers N mineralisation and therefore the emission of  $N_0O$  (Davies *et al.*, 2001), which explains the higher global warming potential calculated for the grass dominated temporary grassland than for the permanent grassland. Short term temporary grasslands also negatively affect the eutrophication potential of forage production because of the potentially high NO<sub>3</sub> losses between two crops (Kayser et al., 2008). Nevertheless, when mixtures with high legume proportion (40-50%), which require much lower N fertilization, are used, SALCA predicts that the NO<sub>3</sub> effect on global eutrophication is compensated by the decrease in NH<sub>3</sub> emissions from fertilization (Figure 1e, 182 and 76 kg N ha<sup>-1</sup> yr<sup>-1</sup> applied in the grass dominated and the legume rich mixtures, respectively). In the experiment of Nyfeler et al. (2011), grass-clover mixtures with up to 50-60% legumes took up at least as much N from soil and fertilizer as grass monocultures did, although the clover fixed large amounts of N. Correspondingly, the NO<sub>3</sub> content in soil water was not elevated under grass-clover mixtures as long as the relative clover abundance was below 80%. Scherer-Lorenzen et al. (2003) reported higher NO<sub>3</sub> leaching under grass-legume mixtures than under grass monocultures in an unfertilized system, despite a significantly higher biomass production in the mixtures. At similar levels of biomass production, and therefore higher N applications on grass monocultures, NO3 leaching from grass-clover swards has been reported to be in the range of those of grass monocultures (Ledgard *et al.*, 2009). Temporary grasslands are directly competing with other arable crops, while this is the case for only parts of the permanent grassland due to soil or climate conditions. In general more digestible energy for human nutrition can be produced by arable crops than by grassland used by ruminants. However, temporary grasslands have an important function in the crop rotation for the maintenance of soil organic carbon (Griffiths et al., 2010), weed control, increasing earthworm populations, etc. Moreover, the possibility to achieve high legume proportion in temporary grasslands can improve protein self-sufficiency at the farm scale (Peyraud et al., 2009) and therefore reduce the need of overseas import of concentrates (soya) and its associated environmental impacts.

#### Impact of temporary versus permanent grasslands on biodiversity

Plant diversity of permanent grasslands is almost always greater than the diversity of temporary grasslands. Plot species richness of the French national database of permanent grasslands (n = 4300 plots) recorded in eFLORAsys (Plantureux and Amiaud, 2010) shows a mean value of 32 vascular species per plot, with a minimum of 8 and a maximum of 76. The most complex temporary grasslands include 10 to 15 sown species, and up to 10 to 20 other species, mainly weeds or species occurring from the soil seed bank. This comparison nevertheless does not take differences in management intensity between permanent and temporary grasslands into account. Seed mixtures for the sowing of species-rich meadows have been developed in Switzerland with up to 47 species (Suter *et al.*, 2008). Moreover, plant diversity of temporary grasslands is more diverse than pure forage crops, and several animal taxa benefit from this diversity and from the pluriannuality of the production. Temporary grasslands with high legume content may be very interesting for pollinator insects, if the grassland is not harvested before flowering. Grass-clovers and lucerne are also known to be beneficial for field breeding birds like Skylark, Grey Partridge, and Quail, and also for Brown Hare (Kragten *et al.*, 2008).

#### Conclusions

Although management intensity is the main lever to improve the area-related environmental impact of grassland-based production, its effect on the product-based impact is manifold. Both intensive and extensive systems showed advantages from the point of view of product-based impacts, depending on the systems compared. Extensification is always positive for farmland biodiversity, but if a certain level of production has to be maintained, intensive systems that set aside a portion of the area to maintain species-rich grasslands are to be preferred to systems that manage all their grassland plots at medium intensity. Besides management intensity, nutrient management was identified as the main lever to mitigate the environmental impact of grassland-based agricultural production on air, soil and water. Research and implementations targeting efficient nutrient cycling are therefore of utmost importance. At the farm level, decisions concerning nutrient management interact with management intensity and selection of the botanical composition of temporary grasslands. Forage conservation per se and the type of forage conservation do not have major effects on the total impact of the production on air, soil and water, as long as highly resource-consuming conservation processes like barn hay drying are avoided. This does not apply to biodiversity, for which the earliness of the first utilisation and the machinery used are of great importance. Grazing showed clear trade-offs with barn-feeding, which highlights the need for situational decision-making concerning these two systems. An interesting impact of grazing is the creation of heterogeneity which provides opportunities for the maintenance of more plant species and animal wildlife. Short term temporary grasslands negatively affect the environmental performances of forage production compared to permanent grasslands. Nevertheless, temporary grasslands allow to a great extent the selection of botanical composition by selecting an appropriate seed mixture. If botanically well balanced, productive grass-legume mixtures maintained for three years or more are used to substantially reduced nitrogen applications, the product-related environmental performance of temporary grassland is clearly improved and is in the same range as that of intensively managed permanent grasslands. Grass-clover temporary grasslands may also be favourable to animal wildlife, despite of poor plant diversity.

Environmental impacts of grassland management finally appear as quite complex, and thus holistic analyses should be further developed. Mitigation options improving one impact can aggravate another one, as shown for N emissions or for the effects of fertilizer types. Such trade-offs must be depicted to reach well-informed decisions. A process that is insignificant for most of the impact categories can be relevant for one specific impact, as discussed for the type of mower and its impacts on insect taxa. Conclusions drawn from analysis at the plot scale should not be extrapolated to draw conclusions at the farm scale without detailed analysis. This is clearly shown for grazing, for which the strengths and weaknesses identified at the plot scale are not identical with those at the farm scale. Global assessments, by models, indicators or LCA, are therefore essential. Theoretical inputs from ecology can be useful to better understand and model environmental impacts of grassland farming, and multidisciplinary research works is still necessary to increase our knowledge on the impacts of management factors, including the study of interactions, to continue improving the models used for such assessments.

## References

Basset-Mens C., Ledgard S. and Boyes M. (2009) Eco-efficiency of intensification scenarios for milk production in New Zealand. *Ecological Economics* 68, 1615–1625.

Batary P., Orci K.M., Baldi A., Kleijn D., Kisbenedek T. and Erdos S. (2007) Effects of local and landscape scale and cattle grazing intensity on Orthoptera assemblages of the Hungarian Great Plain. *Basic and Applied Ecology* 8, 280–290.

Baumgartner D.U., Mieleitner J., Alig M. and Gaillard G. (2011) Environmental profiles of farm types in Switzerland based on LCA. In: Finkbeiner M. (ed.) *Towards Life Cycle Sustainability Management*, Springer, Dordrecht a.o., pp. 291–300.

Benton T.G., Vickery J.A. and Wilson J.D. (2003) Farmland biodiversity: is habitat heterogeneity the key? *Trends in Ecology and Evolution* 18, 182–188.

Boschi C. and Baur B. (2007) Effects of management intensity on land snails in Swiss nutrient-poor pastures. *Agriculture Ecosystems and Environment* 120, 243–249.

Britschgi A., Spaar R. and Arlettaz R. (2006) Impact of grassland farming intensification on the breeding ecology of an indicator insectivorous passerine, the Whinchat *Saxicola rubetra*: Lessons for overall Alpine meadowland management. *Biological Conservation* 130,193–205.

Ceulemans T., Merckx R., Hens M. and Honnay O. (2011) A trait-based analysis of the role of phosphorus vs. nitrogen enrichment in plant species loss across North-west European grasslands. *Journal of Applied Ecology* 48, 1155–1163.

Cingolani A.M., Noy-Meir I. and Díaz S. (2005) Grazing effects on rangeland diversity: a synthesis of contemporary models. *Ecological Applications* 15, 757–773.

Critchley C.N.R., Fowbert J.A. and Wright B (2007) Dynamics of species-rich upland hay meadows over 15 years and their relation with agricultural management practices. *Applied Vegetation Science* 10, 307–314. Davies M.G., Smith K.A. and Vinten A.J.A. (2001) The mineralization and fate of nitrogen following ploughing of grass and grass-clover swards. *Biology and Fertility of Soils* 33, 423–434.

de Klein C.A.M. and Ledgard S.F. (2001) An analysis of environmental and economic implications of nil and restricted grazing systems designed to reduce nitrate leaching from New Zealand dairy farms. I. Nitrogen losses. *New Zealand Journal of Agricultural Research* 44, 201–215.

Del Prado A., Misselbrook T., Chadwick D., Hopkins A., Dewhurst R.J., Davison P., Butler A., Schröder J. and Scholefield D. (2011) SIMS<sub>DAIRY</sub>: A modeling framework to identify sustainable dairy farms in the UK. Framework description and test for organic systems and N fertilizer optimization. *Science of the Total Environment* 409, 3993–4009.

Diaz S., Lavorel S., McIntyre S., Falczuk V., Casanoves F., Milchunas D.G., Skarpe C., Rusch G., Sternberg M., Noy-Meir I., Landsberg J., Zhang W., Clark H. and Campbelle B.D. (2007) Plant trait responses to grazing – a global synthesis. *Global Change Biology* 13, 313–341.

Eyre M.D., Luff M.L., Rushton S.P. and Topping C.J. (1989) Ground beetles and weevils (*Carabidae* and *Curculionoidea*) as indicators of grassland management practices. *Journal of Applied Entomology* 107, 508–517.

FAO (2002) World agriculture: towards 2015/2030. Summary report. Food and Agriculture Organization of the United Nations, Rome, pp. 97. http://www.fao.org/docrep/004/y3557e/y3557e00.htm

Fischer M. and Wipf S. (2002) Effect of low-intensity grazing on the species-rich vegetation of traditionally mown subalpine meadows. *Biological Conservation* 104, 1–11.

Flysjö A., Henriksson M., Cederberg C., Ledgard S. and Englund J.E. (2011) The impact of various parameters on the carbon footprint of milk production in New Zealand and Sweden. *Agricultural Systems* 104, 459–469. Foster B.L. and Gross K.L. (1998) Species richness in a successional grassland: effects of nitrogen enrichment and plant litter. *Ecology* 79, 2593–2602.

Fowler S., Frost D. and de Carle C. (eds) (2004) Environmental and biodiversity impacts of organic farming in the hills and uplands of Wales. Organic Centre Wales, c/o Institute of Rural Sciences, University of Wales, Aberystwyth, 104 pp.

Gaillard G. and Nemecek T. (2009) Swiss agricultural life cycle assessment (SALCA): an integrated environmental assessment concept for agriculture. In: Int. Conf. "Integrated Assessment of Agriculture and Sustainable Development, Setting the Agenda for Science and Policy", Egmond aan Zee, The Netherlands. AgSAP Office, Wageningen University, pp. 134–135.

Gerber P., Vellinga T., Opio C., Henderson B. and Steinfeld H. (2010) Greenhouse gas emissions from the dairy sector – a life cycle assessment. FAO, Rome, 98 pp.

Grevilliot F., Krebs L. and Muller S. (1998) Comparative importance and interference of hydrological conditions and soil nutrient gradients in floristic biodiversity in flood meadows. *Biodiversity and Conservation* 7, 1495–1520.

Griffiths B.S., Ball B.C., Daniell T.J., Hallett P.D., Neilson R., Wheatley R.E., Osler G. and Bohanec M. (2010) Integrating soil quality changes to arable agricultural systems following organic matter addition, or adoption of a ley-arable rotation. *Applied Soil Ecology* 46, 43–53.

Haas G., Wetterich F. and Köpke U. (2001) Comparing intensive, extensified and organic grassland farming in southern Germany by process life cycle assessment. *Agriculture Ecosystems & Environment* 83, 43–53.

Hartmann M., Fliessbach A., Oberholzer H.R. and Widmer F. (2006) Ranking the magnitude of crop and farming system effects on soil microbial biomass and genetic structure of bacterial communities. *Fems Microbiology Ecology* 57, 378–388.

Humbert J.Y., Ghazoul J. and Walter T. (2009) Meadow harvesting techniques and their impacts on field fauna. *Agriculture Ecosystems and Environment* 130,1–8.

Hutton S.A. and Giller P.S. (2003) The effects of the intensification of agriculture on northern temperate dung beetle communities. *Journal of Applied Ecology* 40, 994–1007.

IPCC (2006) 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Volume 4: Agriculture, forestry and other land use. IGES, Kanagawa, Japan.

Isselstein J., Jeangros B. and Pavlu V. (2005) Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe – a review. *Agronomy Research* 3, 139–151.

Janssens F., Peeters A., Tallowin J.R.B., Bakker J.P., Bekke R.M., Fillat F. and Oomes M.J.M. (1998) Relationship between soil chemical factors and grassland diversity. *Plant and Soil* 202, 69–78.

Jauregui B.M., Rosa-Garcia R. and Al E. (2008) Effects of stocking density and breed of goats on vegetation and grasshopper occurrence in heathlands. *Agriculture Ecosystems and Environment* 123, 219–224.

Jeanneret P., Baumgartner D., Freiermuth R. and Gaillard G. (2006) Méthode d'évaluation de l'impact des activités agricoles sur la biodiversité. Agroscope FAL Reckenholz, 67 pp. http://www.agroscope.admin.ch/oekobilanzen/01194/.

Joyce C. (2001) The sensitivity of a species-rich flood-meadow plant community to fertilizer nitrogen: the Luznice river floodplain, Czech Republic. *Plant Ecology* 155, 47–60.

Kampmann D., Lüscher A., Konold W. and Herzog F. (2012) Agri-environment scheme protects diversity of mountain grassland species. *Land Use Policy* 29, 569–576.

Kayser M., Seidel K., Müller J. and Isselstein J. (2008) The effect of succeeding crop and level of N fertilization on N leaching after break-up of grassland. *European Journal of Agronomy* 29, 200–207.

Kemp D.R. and Michalk D.L. (2007) Towards sustainable grassland and livestock management. *Journal of Agricultural Science* 145, 543–564.

Kirkham F.W., Tallowin J.R.B., Sanderson R.A, Bhogal A., Chambers B.J. and Stevens D.P. (2008) The impact of organic and inorganic fertilizers and lime on the species-richness and plant functional characteristics of hay meadow communities. *Biological Conservation* 141, 1411–1427.

Kleijn D., Kohler F., Báldi A., Batáry P., Concepción E. D., Clough Y., Díaz M., Gabriel D., Holzschuh A., Knop E., Kovács A., Marshall E. J. P., Tscharntke T. and Verhulst J. (2009) On the relationship between farmland biodiversity and land-use intensity in Europe. *Proceedings of the Royal Society B* 276, 903–909.

Knop, E., Herzog, F. and Schmid, B. (2011) Effect of connectivity between restoration meadows on invertebrates with contrasting dispersal abilities. *Restoration Ecology* 19, 151–159.

Kragten S. and De Snoo G.R. (2007) Nest success of Lapwings *Vanellus vanellus* on organic and conventional arable farms in the Netherlands. *Ibis* 149, 742–749.

Kragten S., Trimbos K.B. and De Snoo G.R. (2008) Breeding skylarks (*Alauda arvensis*) on organic and conventional arable farms in The Netherlands. *Agriculture Ecosystems and Environment* 126, 163–167.

Krauss J., Klien A.-M., Steffan-Dewenter I. and Tscharntke T. (2004) Effects of habitat area, isolation, and landscape diversity on plant species richness of calcareous grasslands. *Biodiversity and Conservation* 13, 1427–1439.

Kruess A. and Tscharntke T. (2002) Contrasting responses of plant and insect diversity to variation in grazing intensity. *Biological Conservation* 106, 293–302.

Langevin B., Basset-Mens C. and Lardon, L. (2010) Inclusion of the variability of diffuse pollutions in LCA for agriculture: the case of slurry application techniques. *Journal of Cleaner Production* 18, 747–755.

Ledgard S., Schils R., Eriksen R. and Luo J. (2009) Environmental impacts of grazed clover/grass pastures. *Irish Journal of Agricultural and Food Research* 48, 109–226.

Luo J., de Klein C.A.M., Ledgard S.F. and Saggar S. (2010) Management options to reduce nitrous oxide emissions from intensively grazed pastures: A review. *Agriculture, Ecosystems and Environment* 136, 282–291. Marini L., Fontana P., Scotton M. and Klimek S. (2008) Vascular plant and Orthoptera diversity in relation to grassland management and landscape composition in the European Alps. *Journal of Applied Ecology* 45, 361–370.

Marriott C.A., Fothergill M., Jeangros B., Scotton M. and Louault F. (2004) Long-term impacts of extensification of grassland management on biodiversity and productivity in upland areas. A review. *Agronomie* 24, 447–461.

MEA, Millennium Ecosystem Assessment (2005) Ecosystems and Human Well-being: Current State and Trends, Volume 1. Hassan R., Scholes R. and Ash R. (eds), Island Press, Washington, 917 pp.

Menzi H., Frick R. and Kaufmann R. (1997) Ammoniak-Emissionen in der Schweiz: Ausmass und technische Beurteilung des Reduktionspotentials. Eidgenössische Forschungsanstalt für Agrarökologie und Landbau, Zürich-Reckenholz, *Schriftenreihe der FAL*, 107 pp.

Minarro M., Espadaler X., Melero V.X. and Suarez-Alvarez V. (2009) Organic versus conventional management in an apple orchard: effects of fertilization and tree-row management on ground-dwelling predaceous arthropods. *Agricultural and Forest Entomology* 11, 133–142.

Mountford J.O., Lakhani K.H. and Holland R.J. (1996) Reversion of grassland vegetation following the cessation of fertilizer application. *Journal of Vegetation Science* 7, 219–228.

Nemecek T., Dubois D., Huguenin-Elie O. and Gaillard G. (2011a) Life cycle assessment of Swiss farming systems: I. Integrated and organic farming. *Agricultural Systems* 104, 217–232.

Nemecek T., Huguenin-Elie O., Dubois D., Gaillard G., Schaller B. and Chervet A. (2011b) Life cycle assessment of Swiss farming systems: II. Extensive and intensive production. *Agricultural Systems* 104, 233–245. Nguyen T.L.T., Hermansen J.E. and Mogensen L. (2010) Environmental consequences of different beef production systems in the EU. *Journal of Cleaner Production* 18, 756–766.

Nussbaum K. (2008) Energie-Effizienz von kleegras- versus maisbasierten Rindfleischproduktionssystemen im Schweizer Mittelland. Bachelor-Thesis Swiss College for Agriculture, Zollikofen, 60 pp.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E. and Lüscher A. (2011) Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems and Environment* 140, 155–163.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Lüscher A. (2009) Strong mixture effects among four species in fertilized agricultural grassland led to per-sistent and consistent transgressive overyielding. *Journal of Applied Ecology* 46, 683–691.

Oenema O., Velthof G.L., Yamulki S. and Jarvis S.C. (1997) Nitrous oxide emissions from grazed grassland. *Soil Use and Management* 13, 288–295.

Olff H. and Ritchie M.E. (1998) Effects of herbivores on grassland plant diversity. *Trends in Ecology and Evolution* 13, 261–265.

O'Neill B.F., Deighton M.H., O'Loughlin B.M., Mulligan F.J., Boland T.M., O'Donovan M. and Lewis E. (2011) Effects of a perennial ryegrass diet or total mixed ration diet offered to spring-calving Holstein-Friesian dairy cows on methane emissions, dry matter intake, and milk production. *Journal of Dairy Science* 94, 1941–1951.

Peyraud J.L., Le Gall A. and Lüscher A. (2009) Potential food production from forage legume-based-systems in Europe: an overview. *Irish Journal of Agricultural and Food Research* 48, 115–135.

Pilgrim E.S., Macleod C.J.A., Blackwell M.S.A., Bol R., Hogan D.V., Chadwick D.R., Cardenas L., Misselbrook T.H., Haygarth P.M., Brazier R.E., Hobbs P., Hodgson C., Jarvis S., Dungait J., Murray P.J., Firbank L.G. (2010) Interactions among agricultural production and other ecosystem services delivered from European temperate grassland systems. *Advances in Agronomy* 109, 117–154.

Plantureux S. (1996) Biodiversité, type de sol et intensité de l'exploitation des prairies permanentes du plateau lorrain. *Acta Botanica Gallica* 143, 403–410.

Plantureux S. and Amiaud B. (2010) e-FLORA-sys, a website tool to evaluate the agronomical and environmental value of grasslands. *Grassland Science in Europe* 15, 732–734.

Reynolds H.L., Mittelbach G.G., Darcy-Hall T.L., Houseman G.R. and Gross K.L. (2007) No effect of varying soil resource heterogeneity on plant species richness in a low fertility grassland. *Journal of Ecology* 95, 723–733.

Rook A.J. and Tallowin J.R.B. (2003) Grazing and pasture management for biodiversity benefit. *Animal Research* 52, 181–189.

Schellberg J., Möseler B.M., Kühbauch W. and Rademacher I.F. (1999) Long-term effects of fertilizer on soil nutrient concentration, yield, forage quality and floristic composition of a hay meadow in the Eifel mountains, Germany. *Grass and Forage Science* 54, 195–207.

Scherer-Lorenzen M., Palmborg C., Prinz A. and Schulze E.D. (2003) The role of plant diversity and composition for nitrate leaching in grasslands. *Ecology* 84, 1539–1552.

Silvertown J., Poulton P., Johnston E., Edwards G., Heard M. and Biss P.M. (2006) The Park Grass Experiment 1856–2006: Its contribution to ecology. *Journal of Ecology* 94, 801–814.

Sjodin N.E., Bengtsson J. and Ekbom B. (2008) The influence of grazing intensity and landscape composition on the diversity and abundance of flower-visiting insects. *Journal of Applied Ecology* 45, 763–772.

Smith K.A., Jackson D.R., Misselbrook T.H., Pain R.A. and Johnson R.A. (2000) Reduction of ammonia emission by slurry application techniques. *Journal of Agricultural Engineering Research* 77, 277–287.

Smith R.S., Shiel R.S., Millwards D., Corkhill P. and Sanderson R.A. (2002) Soil seed banks and the effects of meadow management on vegetation change in a 10-year meadow field trial. *Journal of Applied Ecology* 39, 279–293.

Suter D., Zanetti S. and Lüscher A. (2008) Seed mixtures to establish species-rich meadows in the Swiss Central Plateau. *Grassland Science in Europe* 13, 174–176.

Sutter M. (2011) Vergleich der Ökobilanz von stall- und weidebasierter Milchproduktion. Schweizerische Hochschule für Landwirtschaft, Bachelor Thesis, 65 pp.

Van der Werf H.M.G. and Petit J. (2002) Evaluation of the environmental impact of agriculture at the farm level: a comparison of twelve indicator-based methods. *Agriculture, Ecosystems and Environment* 93, 131–145. Wallis de Vries M.F., Parkinson A.E., Dulphy J.P., Sayer M. and Diana E. (2007) Effects of livestock breed and grazing intensity on biodiversity and production in grazing systems. 4.Effects on animal diversity. *Grass and Forage Science* 62, 185–197.

Zechmeister H.G., Schmitzberger I., Steurer B., Peterseil J. and Wrbka T. (2003) The influence of land-use praticices and economics on plant species richness in meadows. *Biological conservation* 114, 165–177.
## Origin, history, management and plant species composition of grasslands in Central Europe – a review

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## Abstract

Until now, relatively little attention has been paid to the origin and history of grasslands in Central Europe and to the sources of information that can be used for such study. The aim of this review was to discuss the origin of natural and semi-natural grasslands in Central Europe. Without any written records, grassland history can be studied using the pollen and macroremnant analysis of different sediments and by soil charcoal analysis. An indicator of grasslands is the ratio of non-arboreal/arboreal pollen and species such as *Plantago lanceolata* and *Urtica dioica* in sediments. Pastures can be indicated by *Juniperus communis* pollen or by its charcoals. Insect-pollinated species can be studied using cesspit sediments and vessels in graves, because of their pollen in honey which was used as a sweetener.

In Central Europe, natural grasslands occurred even before the start of agricultural activities in the Early Neolithic (5500 BC), but their area was relatively small and grassland patches were rather fragmentary in the forested landscape. Large-scale enlargement of grasslands can not be expected to have occurred before the Iron Age. The first scythes come from the 5<sup>th</sup> century BC; therefore, hay meadows did not develop before this time. From the start of the agriculture until the 18<sup>th</sup> century, pastures and pasture forests were dominant sources of forage in Central Europe. Large scale enlargement of hay meadows and decline of pastures in many regions occurred from the 18th century, when livestock were predominantly moved into barns because of higher demand for organic fertilizers that were used to increase crop production on arable land. In some mountain areas, there are no records of large-scale deforestation and enlargement of grasslands until the Middle Ages, in the 14<sup>th</sup> century, and the peak of the agriculturally used area was recorded for the period from the 18th to the first half of the 20th century. Mountain grasslands were converted into arable land during the wars and, in contrast, grasslands replaced arable land after the collapse of agriculture in many regions of former communist countries following the change of the political regime in the 1990s. The dynamics of the grassland area reflect the development of human society, because grasslands are an integral part of the cultural landscape in Central Europe.

Keywords: pastures, meadows, pollen analysis, Prehistory, Middle Ages

## Introduction

In terms of their origin, European grasslands can be divided into three broad categories: (1) natural grasslands, predetermined by natural conditions such as shortage of moisture

for forests in steppe regions of Eastern Europe, or by low temperature and by a short vegetation growing season for forest development above the upper timberline in high mountains or in tundra ecosystems (Hejcman *et al.*, 2006); (2) semi-natural grasslands with a wide range of species richness of vascular plants, ranging from 1 to 67 species per 1 m<sup>2</sup> (Hejcman *et al.*, 2010a; Klimeš *et al.*, 2001) and with herbage production per growing season ranging approximately from 1 to 10 t of dry matter per ha (Pavlů *et al.*, 2006; Smit *et al.*, 2008; Hrevušová *et al.*, 2009; Merunková *et al.*, 2012); the existence of semi-natural grasslands is strictly dependent on long-term human activity since the start of agriculture in the period of the Mesolithic-Neolithic transition; (3) improved grasslands, a product of modern intensive agriculture, comprise several sown and highly productive forage grasses of which *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Festuca arundinacea* and *F. pratensis* are the most frequent (Pavlů *et al.*, 2011; Hejcman *et al.*, 2012), together with legumes such as *Trifolium repens* and *T. pratense* (Rochon *et al.*, 2004; Komárek *et al.*, 2010).

Until now, relatively little attention has been paid to the origin and history of grasslands in Central Europe and to the sources of information which can be used for such a study. The aim of this review was therefore to discuss the origin and management of natural and semi-natural grasslands in Central Europe.

#### The Central European phytogeographic province

Europe can be divided into several phytogeographical provinces with different species pools and extend of natural grasslands. According to phytogeographical map by Rivas-Martinez et al. (2004), the Central European province include northeast France, Belgium, Netherland, Denmark, southern Norway and Sweden, Germany, Poland, Czech Republic, Lithuania, Latvia and Estonia. The Central European province offers moderate summers and cold winters, which favours the development of forests with trees adapted to overwintering and competition for light during the vegetation season. Natural grasslands in this province are therefore rare. There is a gradual transition from the eastern continental climatic conditions supporting plant communities of dry, low-productive calcareous grasslands, with the occurrence of species from the eastern continental steppes such as *Festuca vallesiaca*, F. pallens, Adonis vernalis, Linum flavum, Verbascum phoeniceum, Astragalus exscapus, Campanula sibirica, Crambe tataria or Iris pumila. On the other side of the province, in the grasslands in the western part of Central Europe, steppe species gradually disappear and sub-Mediterranean and sub-oceanic species occur, for instance Bromus erectus. Natural heathlands occurred only under specific edaphic conditions on extremely acid and nutrientpoor soils. Together with Nardus stricta grasslands, heathlands with the dominant species of Calluna vulgaris, Luzula campestris, Danthonia decumbens, Carex pilulifera and Potentilla erecta interfere towards the Atlantic and Sub-Atlantic parts of Europe. Atlantic elements common in Central Europe are Cirsium acaule, Lathyrus linifolius and Galium saxatile. Alpine grasslands are confined to the Central European high mountains above the timberline, where the short and cold season of vegetation growth is the essential driver of vegetation development. Most of the alpine species evolved from tertiary lowland species, and others represent relicts from glacial times. Alpine flora of the Carpathians and Central-European Hercynian mountains is due to former glaciation rather evolutionary young and few endemic species are found there.

Dry grasslands on calcareous soils are generally the most species-rich grasslands in Europe (Karlík and Poschlod, 2009). Pärtel *et al.* (1996) recorded 43 vascular plant species per m<sup>2</sup> in the Estonian Alvar limestone grasslands, and Merunková *et al.* (2012) 38 and 40 species per m<sup>2</sup> in the SE part of Czech Republic and Slovakia, respectively. Klimeš *et* 

*al.* (2001) recorded 67 species per m<sup>2</sup> in grasslands in the White Carpathians Mountains at the borderland between the Czech Republic and Slovakia and these grasslands are considered to be the most species-rich grasslands in Europe. Species richness of common grasslands in Central Europe range approximately from 10 to 25 species per m<sup>2</sup> (Pavlů *et al.*, 2003; Hejcman *et al.*, 2010a; Pavlů *et al.*, 2011), but natural and semi-natural grasslands with fewer than 10 species per m<sup>2</sup> can also be recorded, especially on highly acid soils above the upper tree limit (Semelová *et al.*, 2008; Hejcman *et al.*, 2009; 2010b). The extraordinary high species richness of calcareous grasslands can be explained by larger pools of calcicole than calcifuge species, despite the contemporary predominance of acid soils in Central Europe (Chytrý *et al.*, 2003). This disparity in the species pool has resulted from historical and evolutionary processes that took place on high PH soils (Pärtel, 2002). In the Pleistocene, calcareous soils dominated in the dry continental landscapes of Central Europe and in the glacial refugia of temperate flora situated mostly in southern European mountains with abundant limestone and dolomite.

## Pollen analysis and natural grasslands in the forest zone of Central Europe

The occurrence and range of natural grasslands in Central Europe is a topic for discussion which began at the start of the 20th century by "the steppe theory" of Gradmann (1933). According to this theory, the first farmers in Central Europe colonized the steppe grasslands that had survived in the forested landscape from the last glaciation. This theory has been criticised by palynologists. In sediments from the Neolithic the pollen is predominately of forest species (85 – 90% of arboreal pollen, see Kreuz, 2008) with minimal indices for open steppe or grassland vegetation (Margielewski et al., 2010). The problem with pollen analysis is its low sensitivity for indication of grasslands in highly forested landscapes or in heavily grazed forests. Forests serve as "pollen filters" and therefore small areas of natural grasslands in highly forested landscapes can be detected only if the pollen from sediments that accumulated in close vicinity to such grasslands is analysed. Further, there is a substantially higher pollen production by wind-pollinated woody species, especially pine (Pinus spp.) or birch trees (Betula spp.), than by understorey species, from vegetation in clearings, and from other grassland species particularly under grazing management. In heavily grazed forests, pollen production of understorey species is generally low, because of removal of reproductive organs by grazers and presence of insect pollinated species.

A further problem with pollen analysis is the frequent absence of suitable sediments for the preservation of pollen grains in close vicinity of steppe grasslands, and frequently also in close vicinity of densely inhabited Neolithic areas. For example in the Czech Republic, the most suitable sediments for pollen analysis are in peat bogs located in mountains without any Neolithic agricultural activities. Fragmentary steppe grasslands were probably present only in the driest lowland regions of Central Europe and on basic soils, which do not enable good preservation of pollen grains. Low proportions of non-arboreal pollen in analysed sediments cannot, therefore, be regarded as evidence that open steppe and other grassland sites were completely missing from the forested landscape. In the Czech Republic, fragmented areas of steppe grasslands were probably present in lowlands on south-exposed slopes. In such grasslands, the border between steppe and forest vegetation can be very sharp, as is clear from some recent examples from the Czech Middle Mountains. There have been discussions about whether the sharp border between steppe and forest is of natural or human-induced origin. Clearly natural analogies of a sharp border between steppe and forest from the South Ural Mountains or from south Siberia indicate that very sharp borders can be of natural origin (see Horsák et al., 2010). High similarity of "exposition steppes" in the Czech Republic and in Russia indicates that the existence of such grasslands was probably predetermined by natural conditions rather than by human activity, although human activity certainly enabled their enlargement in Central Europe.

Recently, sediments suitable for pollen analysis were discovered close to the current "steppe area" in the Czech Republic and analysed by R. Kozáková (Pokorný, 2011). In this unique profile, the proportion of non-arboreal pollen was higher than 25% even before the start of the Neolithic 5500 years BC. Pollen of *Artemisia* sp., an indicator of open steppe grasslands, was permanently high without any interruption over the last 9000 years. Results clearly indicate natural origin of steppe grasslands in this part of the Central Europe and that typical steppe species survived the development of forests before the start of Neolithic agricultural activities.

## Analysis of macroremains and steppe grasslands

In addition to pollen analysis, long-term existence of steppe grasslands in Central Europe can be indicated by macroremains of steppe species recorded during archaeological excavations of prehistoric sites. Such records are extremely rare, but they exist in Central Europe. In north-east Austria for example, remnants of Stipa pennata, Teucrium chamaedrys, Asperula cynanchica and Plantago media were recorded in burnt houses of Late Neolithic Baden and Jenišovice Cultures (3600-2800 BC, Kohler-Schneider and Caneppele, 2009). In the central part of the Czech Republic, well-preserved remnants of Stipa sp. (feather grass) were recorded in the storage pit of a Unětická Culture from the Early Bronze Age (2300–1600 BC, Bieniek and Pokorný, 2005). Well-preserved awns of Stipa sp. were recorded in the storage pit in very high quantities, indicating their intentional gathering probably for decorative or fire-making purposes. In addition, caryopses of *Stipa* spp. are considered to be edible and they were probably intentionally collected as food. Remnants of Stipa clearly indicate there was a large area of steppe grasslands in this part of the Czech Republic during the Bronze Age. In central Poland, macroremains of Stipa pennata s. l. were recorded in large quantities in Neolithic settlements of the Linear Pottery (syn. Linienbandkeramic - LBK, 5400–5000 BC) and Lengyel (4400–4000 BC) cultures in the Kujawy region (Bieniek, 2002). The presence of this xerothermic grass in archaeological situations can be explained by gathering of this plant and by the presence of steppe grasslands in this part of the Poland during the Neolithic.

We can conclude that at least small-scale steppe grasslands of natural origin were present in the forest zone of Central Europe before the start of agricultural activities in the Neolithic.

## Herbivores and alluvial grasslands

In addition to steppe grasslands, some natural alluvial or wetland grasslands probably existed in Central Europe before the Neolithic. They were predetermined by floods and by the activities of the European beaver (*Castor fiber*). The presence of beaver in Central Europe during prehistory was evidenced by discovery of its skeletal remains (Komosa *et al.*, 2007) and by characteristically cut trees (Pokorný, 2011). Beavers build dumps on shallow rivers, which increase the water table substantially and therefore the surrounding alluvial forests become waterlogged. Trees die in waterlogged conditions, and the result is treeless alluvial grassland after destruction of the dam. In addition to the effects of increasing the water table, the beaver also prevents forestation by direct cutting of trees and by eating bark of standing trees, thus increasing their mortality. Because of beaver activity, strips of alluvial grasslands can be assumed to have developed around rivers even without any human activity. Alluvial grasslands are well supplied by nutrients and by water, and therefore

they produce large amounts of herbage of relatively high quality during the whole vegetation season (Hrevušová et al., 2009). We can therefore suppose that there was selective and intensive grazing by large herbivores on these grasslands, which would have prevented their forestation. In addition to beavers, large herbivores such as wild horses (*Equus* sp.), roe deer (Capreolus capreolus), red deer (Cervus elaphus), aurochs (Bos primigenius) and European bison (Bison bonasus) were present in prehistoric forests and they could maintain open forests or treeless plots with dominant grasses by grazing, and in winter time by browsing on trees and shrubs (Vera, 2002). Hunting of large herbivores was common during the Neolithic, indicating their high occurrence in the landscape. Kyselý (2005) investigated the occurrence of wildlife bones on 39 Neolithic localities in the Czech Republic. The most frequently hunted animal was red deer, recorded on 69% of studied archaeological localities, followed by roe deer (41%), aurochs (38%), hare (Lepus europeus, 36%), wildboar (Sus scrofa, 36%) and beaver (23%). Although the importance of the effect of large herbivores on the presence of open prehistoric forests has been much criticised (see Mitchell, 2005; Kreuz, 2008), it is clear that large herbivores were able to open the forests in places with their high concentration.

We personally studied behaviour and grazing ecology of European bison in the Cherga Nature Reserve (Russia, Altai Mountains) in summer 2011. Almost every tree of Betula pendula or Larix sibirica was damaged by scratching, resulting in their high mortality. Although the total density of animals was low in the reserve, all animals concentrated into a small part of the reserve where they substantially opened the forest. We learned that for the development of open forest vegetation with the presence of grassland species, it is more important that animals are able to build up herds and to concentrate into particular places where they heavily affect forest vegetation, rather than achieving a particular mean density of animals per total area of the reserve. In winter time, bison stripped shrubs and trees and browsed twigs if left for several days without any supplementary feeding. The most preferred woody plants were Salix sp. and Betula pendula. A similar experience with winterfeeding of bison was recorded in the Białowieza primeval forest in Poland by Kowalczyk et al. (2011). The amount of woody materials (trees and shrubs) consumed by bison increased with decreasing access to supplementary fodder, ranging from 16% in intensively fed bison to 65% in non-fed bison utilising forest habitats. Conversely, the amount of herbs, grasses and sedges decreased from 82% in intensively fed bison to 32% in non-fed bison utilising forest habitats. The woody species that were browsed by bison were mainly Carpinus betulus, Corylus avellana, Betula sp. and Salix sp. We personally recorded similar feeding behaviour and stripping of trees in winter enclosures with red and roe deer, and also with Highland cattle, in the Giant Mountains. Finally, we concluded that large herbivores were able to open *Ouercus* forests in Central Europe, but not equally in all sites. They were able to maintain fragments of grasslands in places where they concentrated. Such small-scale grasslands, which can be hardly detected by pollen analysis, probably enabled the survival of grassland species in a generally forested landscape.

## Alpine grasslands

The next group of natural grasslands are Alpine grasslands. These grasslands are common in areas above the upper tree limit in large mountain regions such as the Carpathians and the Alps. A small area of alpine grasslands can also be recorded in the Giant Mountains. Although alpine grasslands are considered to be natural in the Giant Mountains, they were substantially enlarged by agricultural activities from the  $16^{th}$  to the  $19^{th}$  century pushing the upper tree limit down to lower altitudes (Hejcman *et al.*, 2006). Finally, we concluded that, historically, there have been three main categories of natural grasslands in Central Europe: fragmentary steppe grasslands in lowland regions especially on south exposed slopes, alluvial grasslands maintained by beaver-induced deforestation and by the subsequent grazing by large herbivores, and alpine grasslands situated above the upper tree limit.

#### Origin of semi-natural grasslands in Central Europe

It is likely that Central Europe was densely forested in the Holocene climatic optimum (also called Atlantic period, 7500-4000 BC). There are also indications of differences between fully forested regions and areas of semi-opened landscape. The "virgin forest" cannot be perceived as a closed canopy cover, but more or less as an open mixture of woodland with scattered islands of small steppe-like areas. This concept was developed by Ložek (1973; 1981) on the basis of malacostratigraphic data, postulating a continuity of xerothermic herbaceous vegetation in some Czech regions. In the Holocene climatic optimum, important changes in the natural forest composition are recorded (Beneš, 2004). At all altitudinal zones, previous pine and/or birch-dominated woodlands were replaced by a new set of forest trees. In the lowlands, mixed oak woodlands developed, characterised by the occurrence of broadleaf trees (Quercus, Tilia, Ulmus, Corylus, Fraxinus) (Pokorný, 2004). How open the woodland actually was, and how much energy was spent by humans for its initial clearing, is still being researched. The origin of semi-natural grasslands should be connected with the presence of Neolithic settlers (LBK culture, 55004800 BC), and with their preference for loess-based soils. Loess is a glacial, calcium-rich sediment, that is present especially in the Czech lowlands. Although Czech LBK sites generally correspond with loess-based soils, this correlation is not quite strict. It is little realised that the distribution of LBK sites in the Czech Republic more accurately reflects a relationship with easily tilled soils, rather than the fertile but heavy chernozem soils. In younger Neolithic periods (Stroked Pottery (4800-4000 BC) and Lengyel cultures (4300-4000 BC)), there is a higher tendency to occupy heavier soils (Rulf, 1983). It is uncertain whether these soil preferences reflect the actual settlement choices of the earliest farmers, or if this process indirectly records the expansion of secondary anthropogenic grassland, and therefore an increase in the development of chernozem soils. The second possibility seems more probable (Beneš, 2004). The start of agricultural activities can be detected by a decrease in the proportion of pollen of woody species in sediments and by an increase in pollen of non-arboreal species in forested Central Europe. The presence of arable agriculture is indicated by pollen of cereals, wind-pollinated Secale cereale being the most frequent, and of arable-field weeds from the families Chenopodiaceae, Brasicaceae and Poaceae and determinable weedy species such as Centaurea cyanus or Polygonum aviculare in sediments (Kreuz, 2008; Margielewski et al., 2010). A problem of pollen analysis is the inability to determine many taxons at the level of individual species according to their pollen; therefore, in many cases, the pollen analysis must be supplemented by other methods such as macroremnants or phytolite analyses to determine the presence of individual plant species in the landscape. A species with easily determinable pollen is *Plantago lanceolata* and this species is considered to be a good indicator of semi-natural grasslands in the landscape (Poschlod and Baumann, 2010; Brun, 2011). An advantage of P. lanceolata is its occurrence in all types of grasslands irrespective of biomass production and soil pH. In the Rengen Grassland Experiment (SW Germany) for example, P. lanceolata was the only forb species recorded in all fertilizer treatments, from the low productive Nardus stricta grassland with annual herbage production 3 t ha<sup>-1</sup> in the control up to the tall grass community under N, P and K application with dominant *Arrhenatherum elatius* and with herbage production over 10 t ha<sup>-1</sup> (Hejcman *et al.*, 2010a).

Using charcoals of woody species in the soil profile together with pollen analysis of alluvial sediments, Poschlod and Baumann (2010) suppose a continuous existence of dry calcareous grasslands in Franconia (Bavaria, Southern Germany), certainly from Roman times (approximately 30 BC) and most probably since at least the Early Bronze Age (1800 BC). This assumption is based on the presence of charcoals and pollen grains of the light-demanding shrub Juniperus communis. This shrub is typical for open calcareous pastures in Central Europe and cannot survive for a long time in closed forests. In addition, the amount of J. communis pollen in alluvial sediments was well correlated with pollen of typical grassland taxons such as Plantago lanceolata, Galium type, Apiaceae, Ranunculaceae and Ballota/ Galeopsis types. Another indicator of grasslands and ruderal sites in the landscape with identifiable pollen is Urtica dioica. A disadvantage of this species is its occurrence also in alluvial forests and its affinity to soils that are well supplied with N, P and K. Therefore, an increase in pollen of U. dioica in sediments can indicate human settlement activity in the landscape and also intensive livestock grazing. Urtica dioica can become a dominant species within two or three years of increased nutrient availability, as documented by Heicman et al. (2012). According to our personal observations, U. dioica is generally avoided by grazing animals and is consumed only in hay or silage, or as fertile parts of fresh plants in the autumn, and as whole plants after the first winter frosts which interrupt the anti-herbivore function of stinging trichomes. Urtica dioica was a common species in the first agricultural settlements since the LBK Culture in Central Europe, as evidenced by its presence in pollen diagrams and by frequently recorded remnants of seeds in sediments and cultural layers (Margielewski et al., 2010; Out, 2010). The next indicator of grasslands and ruderal sites since prehistory is Trifolium repens-type pollen (a strict determination of T. repens according to its pollen is not possible). As T. repens is an insect-pollinated species, only a relatively small amount of its pollen is frequently recorded in sediments in comparison with that of many other wind-pollinated species (Brun, 2011). From the start of the Neolithic up until the Iron Ages, the area of grasslands and arable fields was relatively small in Central Europe, and forests still predominated, even in the most densely populated lowland regions with loess soils (Pokorný, 2011). Large-scale deforestation is supposed to have started around 1000 BC in Central Europe (Kaplan et al., 2009), causing extensive soil erosion (Beneš, 1995). Despite the forest dominance, it must be noted that many prehistoric settlements were located on the border of present-day extraordinary species-rich calcareous grasslands, indicating their long-term continuity in the landscape since at least the Iron Age (Pärtel et al., 2007; Hájková et al., 2011). In the Czech lowlands, the highest deforestation was recorded after the 13<sup>th</sup> century, when the structure of contemporary settlements was established. The vicinities of many towns were free of forests in the 15<sup>th</sup> century, as was evidenced by low proportion of pollen of arboreal species in anthropogenic sediments collected from the main Czech towns (Beneš et al., 2002; Jankovská, 2011).

## Honey and its use for the study of grassland history

The presence of many insect-pollinated grassland species in the landscape in different historical periods can be studied using anthropogenic sediments in cesspits or vessels in graves originally filled with honey or products made from honey. This is because, from prehistoric times until the 19<sup>th</sup> century, the main sweetener in Europe was honey. The pollen spectrum in honey reflects the spectrum of insect-pollinated species in the landscape and therefore the geographical origin of the honey (Anklam, 1998). After the use of the honey as a foodstuff

the pollen it contains accumulates in faecal sediments, because pollen grains are not destroyed by cooking or by the human digestive tract (Jankovská, 1987). By pollen analysis, the import of honey from floristically different regions can be well identified. For example, Deforce (2010) determined that honey used by the aristocracy in Bruges (Belgium) in the 15<sup>th</sup> century was imported from the western Mediterranean, as cesspit-sediments were rich in the pollen of insect-pollinated species typical for Spain. Calluna vulgaris has frequently been recorded in cesspit sediments, and this species was substantially more common during the Late Middle Age than it is today in the Czech Republic, and indicates the presence of oligotrophic acid pastures in the neighbourhood of big medieval towns (Jankovská, 2011). Food prepared from Avena sativa and sweetened with honey was identified in a ceramic vessel from a grave from the 10<sup>th</sup> century close to Libice above Cidlina river in the Central part of the Czech Republic by Pokorný and Mařík (2006). Honey of local origin was identified according to dominance of insect-pollinated species in the sediment, and according to the presence of immature pollen grains which can be transported only by insects from flowers. Species richness of pollen grains was very high in comparison with recent honey, indicating high diversity of biotopes in the landscape of the 10th century. Taxons indicating the presence of alluvial grasslands were Filipendula, Cirsium, Carduus, Hypericum perforatum type, Centaurea jacea type, Plantago lanceolata, Campanula, Daucus type, Mentha type, Rhinanthus, Asteraceae, Lamiaceae, Serratula type and Peucedanum type. Taxons indicating presence of dry grasslands were Helianthemum, Calluna vulgaris, Brasicaceae, Artemisia, Poaceae, Centaurea scabiosa, Aster type, Rubiaceae, Anthemis type, Plantago media, Gentianella germanica type, Pulsatilla and Sedum. A wide spectrum of identified species indicates that honey was collected in autumn, as both early - and late -flowering species were recorded together. Local origin of the honey was evidenced by the presence of pollen of Nymphoides *peltata*, a water plant that is very rare today and which occurs only in the lowland region of the Czech Republic where the vessel with honey remnants was discovered. It is interesting that the pollen of arable weeds is almost absent, indicating the presence of grasslands and alluvial forests (pollen of Tilia cordata, Alnus glutinosa and Humulus lupulus) in the neighbourhood of the hive. As shown by this unique finding, remnants of honey discovered during archaeological excavations are extremely valuable for study of vegetation history with respect to insect-pollinated plant species, and of grasslands particularly.

#### History of pastures and meadows

According to their management, semi-natural grasslands can be divided into pastures, meadows and grazed meadows. Pastures are managed by livestock grazing, meadows by regular cutting, and grazed meadows are cut in spring and then grazed in summer and/ or in autumn (Hejcman *et al.*, 2010c; Pavlů *et al.*, 2007). We use this clear and simple categorization of semi-natural grasslands according to their management as this terminology is well followed in the agronomic literature, though not in archaeology or ecology. In archaeological or ecological literature, the term "pasture" is frequently used for low-productive grasslands and "meadow" for highly productive grasslands, irrespective of their actual management (see for example Rozbrojová *et al.*, 2010). In Prehistory, only pastures (from the management point of view) and forest pastures existed because there was no tool for grass cutting. The first iron short-scythes suitable for cutting grasslands come from the Iron Age and are dated into fifth century BC (Beranová and Kubačák, 2010). Therefore, in Central Europe, hay meadows could not be established before the late Hallstatt Period (500 BC). Long scythes of today's shape have been used since the Middle Ages. In the Czech Republic, the oldest long scythe comes from the turn of 13<sup>th</sup> and 14<sup>th</sup> century from archaeological excavations of the small castle Bradlo in the NE part of the Czech Republic (Beranová and Kubačák, 2010). Large-scale enlargement of meadows was not recorded until the 18<sup>th</sup> century, when the livestock were moved into barns for the whole year to produce farm yard manure, which was used to increase crop production on arable land in many regions of Central Europe, especially in the Czech Republic (Petrášek, 1972). Large parts of the pasture areas were converted into arable land or into meadows. Remnants of former pasturelands in Central Europe that represent grasslands with a presence of scattered shrubs of *Juniperus communis*, which were common in the past, are present today only in several nature reserves in the Czech Republic or in Germany. Remnants of pasturelands are more common in mountain regions of Slovakia where grazing management is still practised.

## Leaf fodder instead of hay

From Neolithic times up to the Middle Ages, leaf fodder from trees was frequently used instead of grass silage or hay for the winter feeding of livestock. The most valuable woody species for winter fodder production were *Tilia* spp., *Ulmus* spp. and *Corylus avellana* (Sádlo *et al.*, 2005). In addition to woody species, species with winter-green leaves such as *Hedera helix* and *Viscum album* were collected during the winter time and used as fodder for livestock (Deforce *et al.*, 2012). In many regions, a decrease in pollen production of *Tilia* or *Ulmus* in prehistory was linked to the frequent cutting of trees for production of winter forage. Both species regenerate well after coppicing, but the re-sprouting twigs do not flower and therefore their coppicing can be detected by the reduced amount of pollen in sediments.

## Transport of diaspores of grassland species through Europe

A frequently discussed question has been the transport of diaspores of grassland species during the colonisation of new areas and establishment of semi-natural grasslands. The natural transport of diaspores by windstorms was the main driver for the spreading of species over long distances independent of human activities (Sádlo et al., 2005). In addition, since prehistory, there have been intensive movements of people and livestock among the different regions of Europe because of trade and wars. These movements are also associated with epizoochoric and endozoochoric transport of germinable diaspores of many grassland species. For example, we recorded massive germination of Trifolium repens in cattle faeces, indicating the ability of legumes to be transported via endozoochory. There are several methods for estimating historical livestock movement and therefore the potential for transport of diaspores. Viner et al. (2010), using strontium isotopic analysis of Late Neolithic cattle teeth from Durringhton Wall (Wiltshire, Britain), determined that only two of thirteen animals were native in the area, and others came from regions that were at least 100 km distant. Different examples obtained from written historical records include the war expeditions of the Czech Duke Břetislav I (Czech leader in years 1034–1055). Several settlements were relocated, together with their inhabitants and their livestock, from Poland into Czech territory in year 1039 as a result of its successful military attack of Poland. As the people came from the neighbourhood of the Hedče castle (Giecz in Polish, close to Poznaň), they established two villages with names of Hedčany in the eastern and central part of the Czech Republic (Žemlička, 1997). Resettlement of people, seizure of livestock and their transport over long distances was common practice during Middle Age war expeditions, thus enabling massive transport of diaspores of grassland species between different regions. The next source of information is provided by archaeology. Many different objects originating from the Mediterranean area were discovered during archaeological excavations in the Czech Republic and other countries north of the Alps, indicating intensive movement of people, animals and transport of goods though the Alps since at least the Bronze Age (Venclová *et al.*, 2008). There is also evidence that jewellery made from the black sedimentary rock sapropelite, in the third century BC close to Prague (Czech Republic) was transported into Austria, Hungary, Slovakia, Germany and other regions (Blažková *et al.*, 2011). Long-distance transport of goods was connected with animals, especially horses, and therefore with transport of diaspores of grassland species. The next example of livestock and transport of diaspores is transhumance. In southern Germany, shepherding was common in the 19<sup>th</sup> century and the distance between summer pastures in the Swabian Alb and winter pastures in the Lake Constance basin, or the Rhine Valley, was several hundred kilometres (Poschlod and WallisDeVries, 2002). As was demonstrated by Fischer *et al.* (1996), over 8500 diaspores of 85 plant species can be found in the fleece of an individual sheep. Although generally underestimated, livestock movement and transhumance enabled massive transport of diaspores of grassland species.

There are also direct written examples how people intentionally transported grassland species. Together with their cattle, timber-working families resettled from the Alps to the Giant Mountains in the 16<sup>th</sup> century intentionally took *Rumex alpinus* from their fatherland as a vegetable, forage and medicinal plant (Šťastná et al., 2010). Today, R. alpinus is a troublesome grassland weedy species on soils that are well supplied with water and nutrients in the Giant Mountains. Onobrychis viciifolia, a legume native in the Mediterranean, was used as a fodder plant in Central Europe from the 16<sup>th</sup> century (Poschlod and WallisDeVries, 2002). Today, O. vicifolia is considered as a characteristic plant of species-rich calcareous grasslands in many countries north of the Alps. Arrhenatherum elatius is the dominant grass species of two-cut lowland meadows in Central Europe, but the species probably did not occur, or was very rare, in Central Europe before modern times (Poschlod et al., 2009). The large-scale spread of this species was recorded since the 18th century and still continues. This spread was enabled by the decline of grazing management and by an increase in the proportion of hay meadows from the grassland area in Central Europe in the 18th and 19th centuries. In addition, recent spread of A. elatius into species-rich steppe grasslands has been supported by their absence of management, or infrequent defoliation management, together with high nitrogen deposition (Fiala et al., 2011).

## Development of the grassland area from 17<sup>th</sup> century to 2009: an example of a typical mountain village in the Czech Republic

To demonstrate the dynamics of land use in relation to grassland management, the mountain village of Oldřichov v Hájich (Ullersdorf in German) in the Jizera Mountains (Jizerské hory, Góry Izerskie and Isergebirge in Czech, Polish and German) located in the borderland between the Czech Republic, Poland and Germany was selected. This village was chosen because its development in terms of total agricultural area, and proportions of grasslands and arable land, is directly comparable to that of many mountain villages in Central Europe. The first written records about this village come from an urbarium (Urbar in German, a special book for economic survey) from the year 1381. The inhabitants of Oldřichov worked as woodcutters; however, they also kept some cattle and paid a tax from their grasslands. The first written record about the area of agricultural land is from the year 1651, and the number of livestock was counted in 1654 for the first time to provide an economic survey for the payment of taxes. The total agricultural area was approximately 150 ha in year 1651, but over 400 ha was recorded in the period from the second half of the 18<sup>th</sup> century to the first half of the 20<sup>th</sup> century (Figure 1). Then there was a decrease recorded in the area of



Figure 1. Total agricultural area, area of grasslands and area of arable land in village Oldřichov v Hájích in period from 1651 to 2009

agricultural land, and it was approximately 300 ha in 2009. This decrease was as a result of reforestation of marginal grasslands and partly due to an increase in built-up areas.

The peak of arable land area was recorded during the World War I, because of the general shortage of food. During the war, more than 100 ha of grasslands were converted into arable land. In the second half of the 20<sup>th</sup> century, arable fields were gradually grassed down up until the 1980s. A sudden change in the land use occurred after the change of the political regime in 1990s, when a partial (and in many regions, total) collapse of agriculture occurred in former communist countries. At this time, the arable land became completely and naturally grassed down, without sowing any grass-seed mixtures, similar to many other abandoned arable fields in the Czech Republic (Lencová and Prach, 2011) and the number of livestock also decreased dramatically (Figure 2).



Figure 2. Livestock units (one LU is 500 kg of live weight) in village Oldřichov v Hájích over period from 1654 to 2009

Currently, there is the same livestock loading in the study area as there was in year 1651, but the grassland area is six times larger. The majority of grasslands are thus delivering little agricultural production and they have been managed by mulching, infrequent cutting or highly extensive grazing because of state subsidies since the 1990s making their management, even under low livestock loading, profitable. This case study clearly demonstrates that the grassland area in the landscape has been largely affected by the political situation. We have learned that small changes in land use have occurred gradually, but dramatic changes have generally occurred very quickly, over a time scale of one or several years. Grasslands in the landscape were not only established but they were also converted into arable land and naturally or artificially reforested in different historical periods.

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## References

Anklam E. (1998) A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry* 63, 549–562.

Beneš J. (1995) Erosion and accumulation processes in the late holocene of Bohemia, in relation to prehistoric and mediaeval landscape occupation. In: Kuna, M., Venclová, N. (eds), *Wither archaeology ? Papers in honour Evžen Neustupný*, Prague, CZ, pp. 133–144.

Beneš J. (2004) Palaeoecology of the LBK: Earliest agriculturalist and landscape of Bohemia, Czech Republic. In: Lukes A. and Zvelebil M. (eds), LBK Dialogues. *Studies in the formation of the Linear Pottery Culture*. BAR International Series 1304, 143–150.

Beneš J., Kaštovský J., Kočárová R., Kočár P., Kubečková K., Pokorný P. and Starec P. (2002) Archaeobotany of the Old Prague Town defence system, Czech Republic: archaeology, macro-remains, pollen, and diatoms. *Vegetation History and Archaeobotany* 11, 107–119.

Beranová M. and Kubačák A. (2010) Dějiny zemědělství v Čechách a na Moravě, Libri, Prague.

Bieniek A. (2002) Archaebotanical analysis of some early Neolithic settlements in the Kujawy region, central Poland, with potential plant gathering activities emphasised. *Vegetation History and Archaeobotany* 11, 33–40. Bieniek A. and Pokorný P. (2005) A new find of macrofossils of feather grass (*Stipa*) in an Early Bronze Age storage pit at Vliněves, Czech Republic: local implications and possible interpretation in a Central European context. *Vegetation History and Archaeobotany* 14, 295–302.

Blažková K., Sedláček F., Stolz D., Stolzová D. and Venclová N. (2011) Příběh nejkrásnější keltské hlavy – pohled do života Keltů nejen na Novostrašecku. Ústav archeologické památkové péče středních Čech. Prague.

Brun C. (2011) Antropogenic indicators in pollen diagrams in eastern France: a critical review. *Vegetation History and Archeobotany* 20, 135–142.

Chytrý M., Tichý L. and Roleček J. (2003) Local and regional patterns of species richness in Central European vegetation types along the pH/calcium gradient. *Folia Geobotanica* 38, 429–442.

Deforce K. (2010) Pollen analysis of 15<sup>th</sup> century cesspits from the palace of the dukes of Burgundy in Bruges (Belgium): evidence for the use of honey from the western Mediterranean. *Journal of Archaeological Science* 37, 337–342.

Deforce K., Bastiaens J., Van Neer W., Ervynck A., Lentacker A., Sergant J. and Crombé P. (2012) Wood charcoal and seeds as indicators for animal husbandry in a wetland site during the late mesolithic-early neolithic transition period (Swifterbant culture, ca. 4600 – 4000 B.C.). *Vegetation History and Archaeobotany*. DOI: 10.1007/s00334-012-0354-2.

Fiala K., Tůma I. and Holub P. (2011) Effect of nitrogen addition and drought on above-ground biomass of expanding tall grasses *Calamagrostis epigejos* and *Arrhenatherum elatius*. *Biologia* 66, 275–281.

Fischer S., Poschlod P. and Beinlich B. (1996) Experimental studies on the dispersal of plants and animals by sheep in calcareous grasslands. *Journal of Applied Ecology* 33, 1206–1222.

Gradmann R. (1933) Die Steppenheidetheorie. Geographishe Zeitschrift 39/5, 265-278.

Hájková P., Roleček J., Hájek M., Horsák M., Fajmon K., Polák M. and Jamrichová E. (2011) Prehistoric origin of the extremely species-rich semi-dry grasslands in the Bílé Karpaty Mts. (Czech Republic and Slovakia). *Preslia*, 83, 185–204.

Hejcman M., Češková M., Schellberg J. and Pätzold S. (2010a) The Rengen Grassland Experiment: effect of soil chemical properties on biomass production, plant species composition and species richness. *Folia Geobotanica* 45, 125–142.

Hejcman M., Češková M. and Pavlů V. (2010b) Control of Molinia caerulea by cutting management on subalpine grassland. *Flora* 205, 577–582.

Hejcman M., Schellberg J. and Pavlů V. (2010c) Long-term effects of cutting frequency and liming on soil chemical properties, biomass production and plant species composition of Lolio-Cynosuretum grassland after the cessation of fertilizer application. *Applied Vegetation Science* 13, 257–269.

Hejcman M., Klaudisová M., Hejcmanová P., Pavlů V. and Jones M. (2009) Expansion of *Calamagrostis* villosa in sub-alpine *Nardus stricta* grassland: Cessation of cutting management or high nitrogen deposition? Agriculture, Ecosystems and Environment 129, 91–96.

Hejcman M., Pavlů V., Hejcmanová P., Gaisler J., Hakl J. and Rauch O. (2006) Farmer decision making and its effect on the subalpine grassland succession in the Giant Mountains, Czech Republic. *Acta Societatis Botanicorum Poloniae* 75, 165–174.

Hejcman M., Strnad L., Hejcmanová P. and Pavlů V. (2012) Response of plant species composition, biomass production and biomass chemical properties to high N, P and K application rates in *Dactylis glomerata-* and *Festuca arundinacea-*dominated grassland. *Grass and Forage Science.* doi: 10.1111/j.1365-2494.2012.00864.x.

Horsák M., Chytrý M., Pokryszko B.M., Danihelka J., Ermakov N., Hájek M., Hájková P., Kintrová K., Kočí M., Kubešová S., Lustyk P., Otýpková Z., Pelánková B. and Valachovič M. (2010) Habitats of relict terestrial snails in southern Siberia: lessons for the reconstruction of palaeoenvironments of full-glacial Europe. *Journal of Biogeography* 37, 1450–1462.

Hrevušová Z., Hejcman M., Pavlů V., Hakl J., Klaudisová M. and Mrkvička J. (2009) Long-term dynamics of biomass production, soil chemical properties and plant species composition of alluvial grassland after the cessation of fertilizer application in the Czech Republic. *Agriculture, Ecosystems and Environment* 130, 123–130.

Jankovská V. (1987) Netradiční interpretace pylových spekter ze středověké Prahy. Archeologické Rozhledy 39, 435–444.

Jankovská V. (2011) The middle ages in pollen-analytical research on the territory of the Czech Republic. *Bulletin of Geography – Physical Geography Sciences* 4, 47–70.

Kaplan J.O., Krumhardt K.M. and Zimmermann N. (2009) The prehistoric and preindustrial deforestation in Europe. *Quatenary Science Reviews* 28, 3016 – 3034.

Karlík P. and Poschlod P. (2009) History or abiotic filter: which is more important in determining the species composition of calcareous grasslands? *Preslia* 81, 321–340.

Klimeš L., Dančák M., Hájek M., Jongepierová I. and Kučera T. (2001) Scale-dependent biases in 29 species counts in a grassland. *Journal of Vegetation Science* 12, 699–704.

Kohler-Schneider M. and Caneppele A. (2009) Late Neolithic agriculture in eastern Austria: archeobotanical results from sites of the Baden and Jenišovice cultures (3 600 – 2800 BC). *Vegetation History and Archeobotany* 18, 61–74.

Komárek P., Pavlů V. and Hejcman M. (2010) Effect of depth and width of cultivation and sowing date on establishment of red clover (*Trifolium pratense* L.) by rotary slot seeding into grassland. *Grass and Forage Science* 65, 154–158.

Komosa M., Frackowiak H. and Godynicki S. (2007) Skulls of Neolithic Eurasian beavers (Castor fiber L.) in comparison with skulls of contemporary beavers from natural biotopes of Wielkopolska region (Poland). *Polish Journal of Environmental Studies* 16, 697–705.

Kowalczyk R., Taberlet P., Coissac E., Valentini A. and Miquel C. (2011) Influence of management practices on large herbivore diet – Case study of European bison in Białowieża Primeval Forest (Poland). *Forest Ecology and Management* 261, 821–828.

Kreuz A. (2008) Closed forest or open woodland as natural vegetation in the surroundings of Linearbandkeramik settlements? *Vegetation History and Archaeobotany* 17, 51–64.

Kyselý R. (2005) Archaeological evidence of wild mammals in the Czech Republic from the Neolithic to the Modern times. *Lynx*, 36, 55–101.

Lencová K. and Prach K. (2011) Restoration of hay meadows on ex-arable land: commercial seed mixtures vs. spontaneous succession. *Grass and Forage Science* 66, 265–271.

Ložek V. (1973) Příroda ve čtvrtohorách. Academia. Praha,

Ložek V. (1981) Změny krajiny v souvislosti s osídlením ve světle malakologických poznatků – Der Landschaftswandel in Beziehung zur Besiedlung im Lichte malakologischer Befunde. *Archeologické rozhledy* 33, 176–188.

Margielewski W., Krapiec M., Valde-Nowak P. and Zernitskaya V. (2010) A Neolithic yew bow in the Polish Carpathians – Evidence of the impact of human activity on mountainous paleoenvironment from the Kamiennik landslide peat bog. *Catena* 80, 141–153.

Merunková K., Otýpková Z. and Chytrý M. (2012) White Carpathian grasslands: can local ecological factors explain their extraordinary species richness? *Preslia* 84. In press.

Mitchell F.J.G. (2005) How open were European primeval forests? Hypothesis testing using palaeoecological data. *Journal of Ecology* 93, 168–177.

Out W.A. (2010) Integrated archaeobotanical analysis: Human impact at the Dutch Neolithic wetland site the Hazendonk. *Journal of Archaeological Science* 37, 1521–1531.

Pärtel M. (2002) Local plant diversity patterns and evolutionary history at the regional scale. *Ecology* 83, 2361–2366.

Pärtel M., Helm A., Reitalu T., Liira J. and Zobel M. (2007) Grassland diversity related to the Late Iron Age human population density. *Journal of Ecology* 95, 574–582.

Pärtel M., Zobel M., Zobel K. and van der Maarel E. (1996) The species pool and its relation to species richness: evidence from Estonian plant communities. *Oikos* 75, 111–117.

Pavlů L., Pavlů V., Gaisler J., Hejcman M. and Mikulka J. (2011) Effect of long-term cutting versus abandonment on the vegetation of a mountain hay meadow (Polygono-Trisetion) in Central Europe. *Flora* 206, 1020–1029. Pavlů V., Hejcman M., Pavlů L. and Gaisler J. (2003) Effect of rotational and continuous grazing on vegetation of the upland grassland in the Jizerské hory Mts., Czech Republic. *Folia Geobotanica* 38, 21–34.

Pavlů V., Hejcman M., Pavlů L. and Gaisler J. (2007) Restoration of grazing management and its effect on vegetation in an upland grassland. *Applied Vegetation Science* 10, 375–382.

Pavlů V., Hejcman M., Pavlů L., Gaisler J. and Nežerková P. (2006) Effect of continuous grazing on forage quality, quantity and animal performance. *Agriculture, Ecosystems and Environment* 113, 349–355.

Pavlů V., Schellberg J. and Hejcman M. (2011) Cutting frequency vs. N application: effect of a 20-year management in *Lolio-Cynosuretum* grassland. *Grass and Forage Science* 66, 501–515.

Petrášek F. (1972) Geneze chovu skotu v českých zemích. Academia, Prague.

Pokorný P. (2004) Postglacial Vegetation Distribution in the Czech Republic and its Relationship to Settlement Zones: Review from Off-Site Pollen Data. In: M. Gojda (ed.) Prehistoric Settlement Patterns in Bohemia – The Potential of Non-Destructive Archaeology. Prague, Academia.

Pokorný P. (2011) Neklidné časy – kapitoly ze společných dějin přírody a lidí. Dokořán, Prague. 370 pp. Pokorný P. and Mařík J. (2006) Nález zbytku medem slazené potraviny ve výbavě raně středověkého hrobu v Libici nad Cidlinou – Kaníně: zhodnocení nálezu z hlediska rekonstrukce krajiny a vegetace. *Archeologické rozhledy*, 68, 559–569.

Poschlod D. and WallisDeVries M.F. (2002) The historical and socioeconomic perspective of calcareous grasslands – lessons from the distant and recent past. *Biological Conservation* 104, 361–376.

Poschlod P. and Baumann A. (2010) The historical dynamics of calcareous grasslands in the central and southern Franconian Jurassic mountains: a comparative pedoantracological and pollen analytical study. *The Holocene* 20, 13–23.

Poschlod P., Baumann A. and Karlík P. (2009) Origin and development of grasslands in Central Europe. In: Veen P., Jefferson R., de Smidt J., van der Straaten J. (eds), *Grasslands in Europe of high nature value*. KNNV Publishing.

Rivas-Martinez S., Penas A. and Diaz T. (2004) Biogeographic map of Europe. Cartographic Service, University of León, Spain.

Rochon J.J., Doyle C.J., Greef J.M., Hopkins A., Molle G., Sitzia M., Scholefield D. and Smith C.J. (2004) Grazing legumes in Europe: a review of their status, management, benefits, research needs and future prospects. *Grass and Forage Science* 59, 197–214.

Rozbrojova Z., Hájek A. and Hájek O. (2010) Vegetation diversity of mesic meadows and pastures in the West Carpathians. *Preslia* 82, 289–306.

Rulf J. (1983) Přírodní prostředí a kultury českého neolitu a eneolitu, Památky archeologické 74, 35–95.

Sádlo J., Pokorný P., Hájek P., Dreslerová D. and Cílek V. (2005) Krajina a revoluce – významné přelomy ve vývoji kulturní krajiny českých zemí. Malá Skála, Prague.

Semelová V., Hejcman M., Pavlů V., Vacek S. and Podrázský V. (2008) The Grass Garden in the Giant Mts. (Czech Republic): residual effect of long-term fertilization after 62 years. *Agriculture, Ecosystems and Environment* 123, 337–342.

Smit H.J., Metzger M.J. and Ewert F. (2008) Spatial distribution of grassland productivity and land use in Europe. *Agricultural Systems* 98, 208–219.

Šťastná P., Klimeš L. and Klimešová J. (2010) Biological flora of Central Europe: *Rumex alpinus* L. *Perspective in Plant Ecology, Evolution and Systematics* 12, 67–79.

Venclová N., Drda P., Michálek J., Militký J., Salač V., Sankot P. and Vokolek V. (2008) Archeologie pravěkých Čech – doba Laténská. Archeologický ústav AV ČR, Praha.

Vera F.W.M. (2002) Grazing ecology and forest history. Oxford University press, New York.

Viner S., Evans J., Albarella U. and Pearson M.P. (2010) Cattle mobility in prehistoric Britain: strontium isotope analysis of cattle teeth from Durrington Walls (Wiltshire, Britain). *Journal of Archaeological Science* 37, 2812–2820.

Žemlička J. (1997) Čechy v době knížecí. Nakladatelství lidové noviny. Prague.

## Session 4.1.

Impact of grassland on air, soil and water

# Product carbon footprint milk from pasture – and from confinement-based dairy farming

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## Abstract

The product carbon footprint (PCF) of milk was calculated for two contrasting, well-managed dairy farms of Northern Germany: a low-input, pasture-based system (PS) and a high-input, confinement-based system (CS). The PCF milk of each farm was quantified by combining a life-cycle-assessment method with field-level measurements. Results presented here are preliminary calculations considering only the currently available dataset of 2010 (2011 is still in progress). Milk produced in the PS had lower PCF than milk from the CS. Excluding the effect of land use on soil C sequestration resulted in PCFs of 0.88 vs. 0.97 kg  $CO_{2eq}$  kg<sup>-1</sup> ECM for the PS and CS, respectively. However, inclusion of C-balances linked to forage production, pasturing, and land-use change significantly improved and worsened the PCF of PS and CS, respectively, i.e. 0.49 vs. 1.15 kg  $CO_{2eq}$  kg<sup>-1</sup> ECM.

Keywords: carbon footprint, dairy farming, global warming potential, greenhouse gas

## Introduction

Schleswig-Holstein is the most northern federal state of Germany and a major European dairy region. Grassland and forage crops are important land uses, accounting for more than 50% of the total agricultural area (MLUR, 2011). In recent decades, dairy farming in Northern Germany has been subjected to ongoing intensification. Consequently, pasturing has become less important in favour of high-input confinement systems. Furthermore, silage maize increasingly replaces grass in cattle diets. It is expected that a further increase in the area of maize cultivation will occur, at least partly at the expense of grassland area. Such land-use change is likely to induce losses of soil C. Dairy farming is reported to be a significant contributor to agricultural greenhouse gas (GHG) emissions. The extent to which dairy farming contributes to the anthropogenic greenhouse effect is largely determined by the management and may additionally vary according to environmental conditions. However, there is still limited knowledge about emissions and mitigation of GHGs in German dairy farming in general, and about the effect of management in particular. The present study therefore aims to answer the following questions: a) How does the management system affect the product carbon footprint (PCF) of milk?; b) What are the main sources of GHGs in the milk production chain?; and c) Which are the most promising GHG mitigation options? In order to answer these questions we quantified the PCF for two contrasting, wellmanaged dairy farms in Northern Germany.

## Materials and methods

GHGs of two contrasting dairy farms were quantified along the milk production chain, from 'cradle to farm gate,' by combining a life-cycle-assessment method (ISO 14040) with field-level measurements. Emissions of  $CO_2$ ,  $CH_4$  and  $N_2O$  were multiplied by their global warming potential 1, 25, and 298, respectively (100-year time horizon), summed up and, finally, referred to the functional unit (1 kg of ECM) to determine the PCF of milk for two

dairy farms: a) a pasture system (PS) with year-round rotational grazing and an annual use of 200 kg of concentrates to produce on average 5900 kg milk cow<sup>-1</sup> year<sup>-1</sup>; and b) a confinement system (CS) with year-round indoor housing and an annual use of 3500 kg of concentrates to produce 11200 l milk cow<sup>-1</sup> year<sup>-1</sup>. GHGs from forage areas and pastures were measured at the field-level. Both farm-level GHG emissions associated with animal and manure management and upstream-chain or pre-farm GHG emissions associated with the input and use of resources were estimated by using operating data of farms and standard emission factors (Table 1). The effect of land use on soil C on-farm and off-farm has also been taken into account: a) on-farm by calculating field-scale C balances; b) off-farm by estimating land use change induced C losses (Table 1). The economic option was chosen to allocate GHG emissions to outputs of milk and meat, i.e. 18 and 11% reduction of PCFs for PS and CS, respectively.

Sources	Greenhouse gas	Method	Reference
ON-FARM (field-level)			
<ul> <li>Forage production (maize, permanent grassland, ley)</li> </ul>	CH <sub>4</sub> , N <sub>2</sub> O CO <sub>2</sub>	Measured Estimated (C balance)	closed chamber method VDLUFA, 2004
- Pasture (grass/clover leys)	CH <sub>4</sub> , N <sub>2</sub> O CO <sub>2</sub>	Measured Estimated (C balance)	closed chamber method VDLUFA, 2004
ON-FARM (farm-level)	÷		
- Enteric fermentation	$CH_4$	Estimated (EF*)	Kirchgessner et al., 1991
- Manure management	$CH_4$	Estimated (EF*)	Clemens <i>et al.</i> , 2006 IPCC, 2006
- Use of fossil fuels/electricity	CO <sub>2</sub> , CH <sub>4</sub> , N <sub>2</sub> O	Estimated (EF*)	Patyk and Reinhardt, 1997
OFF-FARM (upstream chain)			
<ul> <li>Supply of resource inputs (fertilizer, seeds, pesticide, fossil fuels/electricity, concentrates)</li> </ul>	CO <sub>2</sub> , CH <sub>4</sub> , N <sub>2</sub> O	Estimated (EF*)	Biskupek <i>et al.</i> , 1997; Patyk and Reinhardt, 1997; BfL, 2000; Eriks- son <i>et al.</i> , 2005
<ul> <li>Land use change**</li> </ul>	CO	Estimated (EF*)	FAO, 2010

Table 1. Calculation basis for estimating the product carbon footprint (PCF) milk

EF - emission factor; \*\* Soil Closses associated with soybean cultivation in South America (FAO, 2010).

#### **Results and discussion**

Milk of PS and CS was similarly burdened if neglecting C sequestration potential and land use change (PS: 1.08 and 0.88 kg  $CO_{2eq}$  kg<sup>-1</sup> ECM before and after allocation, respectively; CS: 1.08 and 0.97 kg  $CO_{2eq}$  kg<sup>-1</sup> ECM before and after allocation, respectively). However, milk from PS had much lower PCF than milk from the CS if C sequestration potential of pastures and land use change induced C losses were included (PS: 0.60 and 0.49 kg  $CO_{2eq}$  kg<sup>-1</sup> ECM before and after allocation, respectively; CS: 1.29 and 1.15 kg  $CO_{2eq}$  kg<sup>-1</sup> ECM before and after allocation, respectively) (Figure 1). The PCF values of milk were predominantly determined by enteric CH<sub>4</sub> emissions and field-level N<sub>2</sub>O emissions in the PS, and by enteric fermentation and the supply and use of resource inputs in the CS (Figure 1). Taking into account the C sequestration of grasslands associated with forage production and pasturing considerably improved the PCFs of milk, i.e. total GHG emissions were offset by 48 and 9% at PS and CS, respectively (Figure 1). However, animal feeding in CS essentially is based on concentrates and, therefore, it was considered necessary to include all GHG emissions related to the supply of concentrate components. The loss of soil C



Figure 1. The product carbon footprint (PCF) milk (kg  $CO_{2eq}$  kg<sup>-1</sup> ECM) of pasture – (PS) and confinement-based (CS) dairy farming before allocation. (\*incl. soil C sequestration potential and land use changes. \*\*excl. soil C sequestration potential and land land use changes)

through changing land uses (grassland or forest to arable land) is particularly associated with soybean cultivation in South America, which significantly worsens the PCF of the high-input farm CS by producing additional 0.34 kg  $CO_{2eg}$  kg<sup>-1</sup> ECM (Figure 1).

#### Conclusions

The investigated dairy farms exhibited large differences in their PCF of milk, not only regarding total amounts of GHGs but also regarding the contribution of GHG sources. Owing to their potential for sequestering atmospheric  $CO_2$  in grassland soil C stocks, pasture-based systems hold the potential to improve the PCF of milk. However, estimation of soil C sequestration still lacks accuracy, and further knowledge and methodological standardization is required to increase significance of estimations and to achieve comparability among systems and studies.

#### References

BfL (2000) Bewertung von Verfahren der ökologischen und konventionellen landwirtschaftlichen Produktion im Hinblick auf den Energieeinsatz und bestimmte Schadgasemissionen. Bundesforschungsanstalt für Landwirtschaft. *Landbauforschung Völkenrode*, Special Issue 211 (In German).

Biskupek B., Patyk A. nad Radtke J. (1997) Daten zur Pflanzenölproduktion. In: Kaltschmitt M., Reinhardt G.A. (eds). *Nachwachsende Energieträger*. Braunschweig/Wiesbaden. 167–225 (In German).

Clemens J., Trimborn M., Weiland P. and Amon B. (2006) Mitigation of greenhouse gas emissions by anaerobic digestion of cattle slurry. *Agriculture, Ecosystem and Environment*, 112. 171–177.

FAO (2010) Greenhouse Gas Emissions from the Dairy Sector. A Life Cycle Assessment. Food and Agricultural Organization of the United Nations. Rome.

ISO 14040 (2006) Environmental management – Life cycle assessment – Principles and framework. International Organization for Standardization. Geneva.

Kirchgessner M., Windisch W., Müller H.L. and Kreuzer M. (1991) Release of methane and carbon dioxide by dairy cattle. *Agribioogical Research*, 44, 2–3.

MLUR (2011) Flächenanteile im Ackerfutterbau. Ministry of Agriculture, the Environment and Rural Areas Schleswig-Holstein, Germany (Download 06.10.2011: http://www.statistik-nord.de/).

IPCC (2006) 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National. Greenhouse Gas Inventories Programme, Eggleston H.S., Buendia L., Miwa K., Ngara T., and Tanabe K. (eds). Published: IGES, Japan.

Eriksson I.S., Elmquist H., Stern S. and Nybrant T. (2005) Environmental Systems Analysis of Pig Production – The Impact of Feed Choice. *Int J LCA* 10 (2). 143–154.

Patyk A. and Reinhardt G. (1997) Düngemittel-, Energie- und Stroffstrombilanzen. Braunschweig/Wiesbaden (In German).

VDLUFA (2004) Humusbilanzierung – Methode zur Beurteilung und Bemessung der Humusversorgung von Ackerland. Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. Bonn (In German).

## Potential effects of the quality of the carbon sources from *Lolium multiflorum* as a catch crop on the availability of soil nitrogen

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## Abstract

Optimizing nitrogen management is essential in sustainable agriculture. The grass Lolium multiflorum is often used as a catch crop because of its rapid growth and high nitrogen (N) demand. As a carbon source, Lolium tissues contain a considerable phenolic concentration and a high content of water soluble carbohydrates. Whereas labile carbon fuels soil microbial activities, phenols (Ph) are known to slow down N mineralization rates in soils, which may diminish the availability of N to the following crops. The aim of this research is to ascertain whether the effects of Ph on soil N are modulated by the availability of easily usable carbon sources, such as sugars. We added Ph and glucose at different concentrations to an agricultural soil and 24 h afterwards we measured N pools, gross rates of mineralization and nitrification, ammonium and nitrate immobilization, and C and N microbial biomass. Phenolic additions affected negatively microbial C biomass, gross N mineralization and soil ammonium pools. High glucose also reduced soil ammonium pools, but increased microbial C biomass and nitrate immobilization. The results suggest that, although both Ph and sugars decrease soil mineral N availability in the short term, they have divergent effects on the microbiota involved in the N cycle, decelerating gross rates of mineralization in the case of Ph and fuelling microbial growth and causing N starvation in the case of sugars. The consequences are further discussed.

Keywords: C inputs, N cycle, gross rates of mineralization, phenolic acids

## Introduction

Cover crops play a key role in sustainable agricultural practices due to their ability to improve soil fertility and to reduce soil erosion and weed pressure. Legumes and grasses are commonly used as cover crops for different purposes. Legume cover crops improve soil fertility since they fix atmospheric N and the residues left for subsequent crops have a high N content, whereas grasses are used because they have high N requirements and hence, mitigate N leaching losses. Specific traits of the cover crops (quality of residues, N requirements and N fixing ability) have different implications for soil N cycling and for subsequent crops; therefore, choosing the appropriate cover species is crucial to meet the required environmental objectives and to avoid interferences with subsequent crops.

*Lolium multiflorum* has interesting characteristics as a winter catch crop due to its rapid growth under low temperatures, its high N demand and its capacity for weed suppression. As a carbon source when incorporated into the soil, *L. multiflorum* herbage has a high sugar content and a considerable amount of phenolic compounds (Ph). Whereas labile sugars may boost soil microbial growth, Ph are very relevant in controlling plant-plant and plant-soil interactions, playing an important role in soil organic matter dynamics and nutrient cycling.

In general, the overall effect of Ph is to decrease N availability either by slowing down gross rates of N mineralization or by increasing N immobilization (Castells *et al.*, 2003).

The aim of this research is to ascertain if the effects of Ph and *L. multiflorum* extracts on soil N availability are modulated by the effect of sugars, and whether the overall response can be expected in field conditions.

## Materials and methods

We established a field trial in Arazuri (northern Spain; 42°48'N, 1°43'W), on agricultural land with a clay-loam soil, low total nitrogen contents (0.18%), and high contents of mineral N (23.91  $\mu$ g g<sup>-1</sup> N–NO<sub>3</sub><sup>-</sup> and 0.64  $\mu$ g g<sup>-1</sup> N–NH<sub>4</sub><sup>+</sup>). The experiment was set up in autumn 2008. We buried 48 PVC cylinders (9 cm diameter and 10 cm high) to the level of the soil surface in a completely randomized design with six replicates. Soil contained in each cylinder was placed in a plastic bag and sprayed with 10 ml of the following eight solutions: (1) ferulic and coumaric acids (50:50; totals phenols 97.4 µg per g of soil, P); (2) fractionated FE extract of L. multiflorum (total phenols 0.35 µg per g soil; the extract was fractionated by solid-phase extraction using a C18 Extra-sep column to retain phenols); (3) unfractionated UE extract of L. multiflorum (total phenols 2.88 µg per g soil); (4) high glucose (1.95 mg C per g soil, HG); (5) low glucose (19.5 µg C per g soil, LG); (6) high glucose with phenols (PHG); (7) low glucose with phenols (PLG); and (8) a control of distilled water (CON). After spraying, the bags with soil were re-buried into the field and incubated for 24 hours. After this period we measured N pools, microbial biomass N and C (chloroform fumigation and direct extraction) and gross rates of nitrification and mineralization (15N pool dilution technique). Data were normally distributed and were analysed using General Lineal Model procedure of PASW 18.0.

## **Results and discussion**

We found a similar additive effect of recalcitrant and labile carbon pools on the availability of N in the soil (Figure 1A). However, despite the similarities in final effects, the underlying mechanisms differed substantially. Labile glucose increased nitrate immobilization (Figure 1C) and microbial C biomass (Figure 1D), which indicated a fuelling effect on microbial growth due to the addition of an easily usable carbon source. In contrast, more recalcitrant phenols tended to decrease the gross rates of mineralization (Figure 1B) and significantly decreased microbial C biomass (Figure 1D), which suggested a deceleration of the microbial processes related to the N cycle.

N availability for plants is driven by microbial populations which dominate the N cycling. We found short-term (24 h) effects on soil N availability caused by rapid shifts in microbial dynamics. To what extent these mechanisms are maintained in the mid term is unknown. In a scenario of continuous inputs of residues, populations might shift to phenolic acid utilising bacteria (Blum *et al.*, 2000) whereas sugars might induce a severe N starvation (Rasul *et al.*, 2009). Both processes would lead to a low availability of soil N for the following crops. However, in a scenario of discontinuous input of residues, as those occurring in agricultural fields, the fuelling effect of sugars and the negative effect of phenols on N mineralisation may occur only in the early stage of plant residue decomposition (Bending *et al.*, 1998). This temporal effect has the interest of avoiding the loss of soil mineral N before the following crop get established, retaining N in organic forms or in soil microbial populations.



Figure 1. Soil ammonium pools, gross rates of mineralisation, nitrate immobilisation and Microbial biomass C under amendments with phenolic acids and/or glucose. C – control, LG – low glucose, P – phenols, PLG – low glucose with phenols, PHG – high glucose with phenols, FE – fractionated extract, UE – non-fractionated extract

#### Conclusions

Plant residues rich in phenols and/or sugars induce rapid responses on soil microbial populations, and lead to a decrease of soil N availability, which ensures a biological retention of N before the establishment of the following crop.

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## References

Bending G.D., Turner M.K. and Burns I.G. (1998) Fate of nitrogen from crop residues as affected by biochemical quality and the microbial biomass. *Soil Biology and Biochemistry* 30, 2055–2065.

Blum U., Staman K.L., Flint L.J. and Shafer S.R. (2000) Induction and/or selection of phenolic acid-utilizing bulk-soil and rhizosphere bacteria and their influence on phenolic acid phytotoxicity. *Journal of Chemical Ecology* 26, 2059–2078.

Castells E. and Penuelas J. (2003) Is there a feedback between N availability in siliceous and calcareous soils and Cistus albidus leaf chemical composition? *Oecologia* 136, 183–192.

Rasul G., Khan A.A., Khan K.S. and Joergensen R.G. (2009) Immobilization and mineralization of nitrogen in a saline and alkaline soil during microbial use of sugarcane filter cake amended with glucose. *Biology and Fertility of Soils* 45, 289–296.

## Balanced phosphorus fertilization on grassland in a mixed grazing and mowing system: a 13-year field experiment

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## Abstract

In the Netherlands the legal phosphorus (P) application standard is decreasing from the year 2006 to 2013, to balanced P fertilization (input equals output). The long term effect of balanced P fertilization on grassland is estimated by simulations, not in experiments. In a field experiment from 1997 to 2009 the development in time of dry matter (DM) yield and P content of grass at balanced P fertilization is compared with positive P surpluses at two N levels on four permanent grassland sites (sand1 and sand2, clay and peat). The plots were grazed and mown. Three P surpluses (0, 10 and 20 kg P ha<sup>-1</sup> yr<sup>-1</sup>) and two N surpluses (180 and 300 kg N ha<sup>-1</sup> yr<sup>-1</sup>) were applied. The mean DM yield at balanced P fertilization was lower than at 20 kg P ha<sup>-1</sup> yr<sup>-1</sup> surplus, 690 kg DM ha<sup>-1</sup> yr<sup>-1</sup> on sand and 526 kg DM ha<sup>-1</sup> yr<sup>-1</sup> on peat. On clay the difference was reversed. The average P content of the grass at a surplus of 20 kg P ha<sup>-1</sup> was 0.3 g P kg<sup>-1</sup> DM higher than at balanced P fertilization. At all locations, except on clay, the P content of grass from balanced P fertilization declined to values below the Dutch standard for grass in dairy cattle rations with silage maize.

Keywords: grassland, grazing, phosphorus, balanced fertilization, dry matter yield, P content

#### Introduction

In the Netherlands farmers are obliged to decrease fertilization, due to the Nitrate Directive of the European Union. From 2006, the nitrogen (N) and phosphorus (P) fertilization is limited. The legal P application standard is decreasing to 2013, to balanced P fertilization (input equals output). Simulations of the long-term effect of balanced P fertilization on grassland in the Netherlands showed that a positive soil surface P balance of 10–20 kg P ha<sup>-1</sup> yr<sup>-1</sup> is necessary to maintain soil fertility and, as a consequence, the production level of grassland (Oenema and Van Dijk, 1994). This is, however, not measured in experiments. To examine the effect of balanced P fertilization, a field experiment was started in 1997. The objectives were development in time of soil fertility, dry matter yield (DMY) and grass composition at balanced P fertilization, compared with positive P surpluses and the interaction with N level, grazed and fertilized with cattle manure and mineral fertilizer. This paper presents the results of DMY and P content of grass from 1997 to 2009 (13 years).

## Materials and methods

The field experiment was carried out on four permanent grassland sites in the Netherlands: sand (sand1 and sand2), young marine clay, and peat soil. At the start (autumn 1996) the P soil fertility was, according to Dutch standards, sufficient for all four locations. In the 0–5 cm layer, organic matter was 5% on sand1 and sand2, 8% on clay and 52% on peat;  $pH_{KCI}$  was 5.6 on sand1 and sand2, 7.7 on clay, 5.0 on peat ("good" according to Dutch standards). At each location six plots were randomly assigned to a combination of P and N surpluses, without replicates. Fertilization levels were aimed to supply surpluses of 0, 10 and 20 kg P ha<sup>-1</sup> yr<sup>-1</sup> (P0, P10 and P20) and 180 and 300 kg N ha<sup>-1</sup> yr<sup>-1</sup> (N180 and N300). Cattle slurry

and mineral P fertilizer were applied in early spring and before the fourth cut, and mineral N fertilizer was applied throughout the whole season. The P10 and P20 surpluses were to be achieved by applying superphosphate or triple-superphosphate. The first and fourth cuts were taken for silage, and the other cuts were grazed by (non-lactating) heifers. Each plot was split into a part to adapt the animal excreta to the mineral composition of the grass and a part to measure and sample. Assessments of DMY and N and P content were determined when grazing or cutting took place. The surpluses were calculated as fertilization minus output in silage cuts and in weight increase of heifers. Grazed and excreted nutrients were considered to be an internal cycle.

The annual DMY and grass P content were statistically analysed with the Restricted Maximum Likelihood (ReML) technique (Harville, 1977), using Genstat. ReML fits a random and a systematic part of a model to the data. The random part contains factors that are not controllable. The systematic part contains factors that can be controlled (like treatments). Nonsignificant interactions (P > 0.05) were deleted from the model. The analysis determined significant effects ( $P \le 0.05$ ) and inclusions of variables in the model quantified the effects. All equations were linear. In order to determine if there was an indication of curvature, year number<sup>2</sup> was tested. The value of year number in the starting year was 1. Sand1 was included in the analysis until 2001 due to a change to organic fertilization on this location.

#### **Results and discussion**

The N surpluses were much lower and P surpluses slightly lower than planned (Table 1). The lower N surplus proved to be close to the application standard for total N on grassland. The ReML analysis for DMY and P content showed that in the random part year.location had a significant contribution, implying different year effects per location.

N-sur- plus planned kg ha <sup>-1</sup>	P-sur- plus planned kg ha <sup>-1</sup>	Nfertiliza- tion kg ha <sup>-1</sup>	Pfertiliza- tion kg ha <sup>-1</sup>	DM yield kg ha <sup>-1</sup>	N con- tent g kg <sup>-1</sup>	P con- tent g kg <sup>-1</sup>	Total N yield kg ha <sup>-1</sup>	Total P yield kg ha <sup>-1</sup>	N- surplus, actual kg ha <sup>-1</sup>	P- surplus, actual kg ha <sup>-1</sup>
180	Mean	238	33	10816	28.3	3.7	307	39.4	125	8.6
300	Mean	370	35	12250	30.6	3.6	374	43.5	234	8.8
Mean	0	304	24	11340	29.5	3.4	335	39.0	182	0.2
Mean	10	304	34	11518	29.3	3.6	337	41.7	180	8.6
Mean	20	304	44	11740	29.6	3.7	348	43.7	177	17.3

Table 1. Treatments and results of P fertilization experiment on grassland (1997-2009)

\* sand1 results taken until 2001.

For DMY in the systematic part the interaction location.soil type was significant, implying that the level of DMY on the two sand locations was different (Table 2, eq. 1).

Table 2. REML estimates for factors affecting DM yield (1000 kg DM  $ha^{-1}$ ) and P content in grass (g P kg<sup>-1</sup> DM)

	DM yield			P content	
Location	constant	β1 (P fert)	β2 (N fert)	Constant	ß1 (P fert)
Sand1	10.387	0.0345	0.002959	3.571	0.006662
Sand2	8.399	0.0345	0.002959	3.571	0.006662
Clay	6.505	-0.0134	0.01719	4.436	-0.00076
Peat	8.531	0.0263	0.005981	3.538	0.005708

The factors N- and P-fertilization, location and the interactions between soil type and N and P fertilization were significant. The response of DMY to N- and P-fertilization was different between soil types, not between sand1 and sand2. Year number<sup>2</sup> was not significant. According to the model the difference in DMY between P0 and P20 was 690 kg DM ha<sup>-1</sup> on sand, 526 kg DM ha<sup>-1</sup> on peat, and – 268 kg DM ha<sup>-1</sup> on clay (Table 2, eq. 1). There was no significant trend in time in this difference as the interaction of P-fertilization. year number was not significant. The negative effect of P-fertilization on clay was thought to be out of line with other results. It is a consequence of a deviated plot. The lower DMY at a lower P-surplus resulted in a significant ( $P \le 0.05$ ; analysis not shown) lower N-yield (Table 1) and therefore a higher N-surplus.

 $DM \ yield = constant_{location} + P - fertilization*\beta1_{soil type} + N - fertilization*\beta2_{soil type} + year \ number* \\ -0.1296 + year \ number*N \ total \ fertilization*0.00066 + \epsilon_{year+year.location} \ (Equation \ 1)$ 

The ReML analysis for P-content of the grass showed that in the systematic part N- and P-fertilization, soil type, year number, P-fertilization.soil type and P-fertilization.year number were significant (Table 2, eq. 2). The two sandy soils showed no significant difference. P-content responded negatively to N-fertilization, probably by dilution as both N levels were harvested at the same time. P-content responded positively to P-fertilization except initially on clay but after two years the response was also positive. The trend over time was the same (significant and negative) for all locations and treatments. It was becoming less negative at a higher P-fertilization. A zero trend would be reached with a P-fertilization outside the experimental treatments. The net trend for P-content in the experiment was negative.

 $P \text{ content of } grass = \text{constant}_{\text{soil type}} + P \text{-fertilization} * \texttt{B1}_{\text{soil type}} + N \text{-fertilization} * -0.00101 + year \text{ number} * -0.07534 + P \text{-fertilization} * year \text{ number} * 0.0004134 + \varepsilon_{\text{year-year.soil type}} (Equation 2)$ 

In the Netherlands the standard for P-content in dairy cattle rations is  $3.5 \text{ g P kg}^{-1} \text{ DM}$  and  $3.3 \text{ g P kg}^{-1} \text{ DM}$  for grass in rations including silage maize (derived from Valk, 2003) which is common in the Netherlands. At all locations, except clay, grass from P0 declined to values below this standard during the experiment.

#### Conclusions

The mean difference between 13 years of balanced P fertilization and a surplus of 20 kg P  $ha^{-1} yr^{-1}$  was 625–750 kg DM  $ha^{-1} yr^{-1}$  on sand and 530 kg DM  $ha^{-1} yr^{-1}$  on peat. There was a negative effect on clay of P surplus, but this was thought to be out of line with other results obtained at the same site. The average P content of the grass at a surplus of 20 kg P  $ha^{-1}$  was 0.3 g P kg<sup>-1</sup> DM higher than at a surplus of 0 kg P  $ha^{-1}$ . The N surplus had a significant negative effect on P content, probably by dilution as both N levels were harvested at the same time. At all locations, except clay, grass from balanced P fertilization declined to values below the standard for grass in dairy cattle rations with silage maize.

#### References

Harville D.A. (1977) Maximum Likelihood approaches to Variance Component Estimation and to related problems. *Journal of American Statistical Association* 72, 358, 320–338.

Oenema O. and Van Dijk T.A. (1994) Fosfaatverliezen en fosfaatoverschotten in de Nederlandse landbouw. Rapport van de technische projektgroep 'Pdeskstudie' (in Dutch).

Valk H. and Beynen A.C. (2003) Proposal for the assessment of phosphorus requirements of dairy cows. *Livestock Production Science* 79, 267–272.

## Effects of wheel damage on peat grasslands at low bearing capacity at high ditch levels

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## Abstract

From the 1960s onwards in the Netherlands, many relatively wet peat lands were deep(er) dewatered to a ditch water level of 50 to 60 cm below the surface to improve dairy agriculture. As a consequence of this deeper drainage, peat degradation took place due to mineralisation, resulting in a relatively rapid land subsidence. An increase of the low ditch level may inhibit subsidence. Although much research has been done on the effects of deep drainage, hardly any research has been done at the effects of increasing ditch water levels. In a six-year trial, the agricultural effects of increasing ditch level were examined. This article discusses the effects of soil damage caused by rutting when slurry is injected under poor bearing conditions in spring. The damage caused by shallow injecting of slurry at a bearing capacity under 4 kg cm<sup>-2</sup> was equal at both ditch levels. When damage occurs it takes place in the wheel tracks. On average, shallow injection of slurry with a manure tank with a capacity of 7 m<sup>3</sup> led to 4.5% yield loss per hectare.

Keywords: DM yield, peat soil, increased ditch level, wheel damage, rutting

## Introduction

To improve the possibilities for dairy farming in (wet) peat areas in the Netherlands, deeper drainage was considered to be a suitable solution. In the 1970s, ditch levels were lowered to over 60 cm below surface. Dry peat resulted in higher greenhouse gas production (Schrier, 2010) and due to the mineralisation the field level became lowered by subsidence. Therefore, in parts of the Netherlands the policy nowadays is to set up higher ditch levels to prevent further subsidence. A higher ditch level has many effects on different aspects of grassland management.

One of the advantages of a low ditch level was drier land with a better bearing capacity, resulting in less damage by trampling or from machinery (Boxem and Leusink, 1978). Not much research has been done in the Netherlands that has especially examined the effect of high and low ditch levels in peat soil areas on machinery wheeling damage to the sward. Therefore, in the years 2007–2010 a field experiment was set up on the Zegveld research farm, situated in a low peat area. The objective of this experiment was to measure yields on plots with and without wheeling damage. Spring is the most critical time for wheeling damage, because the land is still wet from the winter period and spring is the moment to apply slurry.

## Materials and methods

Two permanent grassland plots were split up in two equal parts. Two ditch water levels, -50 and -30 cm below surface, were randomly assigned to the split parts. This resulted in a design of four plots (two paired replicates, each with two ditch levels). To measure the wheeling damage, in early spring of 2007 to 2010 a tractor with a slurry tank drove over two strips in four plots (two with a high level and two with a low level). One strip

was near the ditch and the other in the middle of the plot. The tank had a capacity of 7 m<sup>3</sup> and was three-quarters filled. During the experiment no slurry was applied, so the weight (14 Mg) of the tank did not change. The tyres were 65 cm wide and the injector was 5.20 m. Within and beside each strip, 3 plots each of 6 m were randomly marked as yield plots before the passage of the machinery. The bearing capacity was measured before and after the tractor passage. On all plots 70 kg N ha<sup>-1</sup> was applied as an artificial fertilizer.

The wheeling ('riding') experiment started at the moment the estimated bearing capacity on the plot with the low ditch level ('Dry') was just enough to prevent sward damage. This is marked as the zero point in Figure 1. The first cut was mown with a special mower for experimental research. The fresh yield was weighed and a sample was dried at 103°C to calculate the DM yield. The differences between the DM yield on the high level and low level plots, but also in and beside the machinery riding track were statistically analysed using residual maximum likelihood (REML; Harvill, 1977) using Genstat (version 12).

#### **Results and discussion**

The bearing capacity was measured from about two weeks before the actual wheeling experiment until six weeks after. The differences in bearing capacity during this whole period were statistically tested. The development in time of the bearing capacity is given in Figure 1. In this figure, the level at which no damage is expected is presented as the threshold. This damage threshold is about 4 kg cm<sup>-2</sup> (Beuving *et. al.*, 1989).



There was a significant difference (P < 0.001) in bearing capacity at the moment of the machinery riding (point 0 in the X-axis). The average bearing capacity at this starting point was 3.7 kg cm<sup>-2</sup> at low ditch level (Dry) and 2.7 kg cm<sup>-2</sup> at a high ditch level (Wet). During the period one to four weeks after the machinery riding, the bearing capacity in the track on the 'Wet' plots was significant lower than besides the track. On average the bearing capacity at 'Dry' was significant higher than on 'Wet', and the development in time was also significant different for both levels. There was a significant interaction (P < 0.001) between ditch level, time and place (on the track or beside the track).

The first cut was harvest at a yield of about 4,000 kg DM ha<sup>-1</sup>. This was visually estimated. The DM yield is shown in table 1.

Low ditch lovel ('Dry')	in the track	3,350 <sup>a</sup>
Low ditch level ( Dry )	beside the track	$3,934^{b}$
Average	3,900	
Uigh ditch lovel ('Wat')	in the track	3,129 <sup>a</sup>
High ditch level ( wet )	beside the track	3,871 <sup>b</sup>
Average	3,500	

Table 1. Average DM yield (kg ha-1) over the period 2007-2010 of the first cut after machinery riding

There was no significant effect in the overall DM yield between 'Dry' and 'Wet'. There was a significant difference in DM yield between the tracks and beside the tracks. This effect had an interaction with the ditch level (P < 0.001, LSD = 320). This means that a high ditch level will lead to more damage in the tracks than a low ditch level. The DM yield in the track was 17% lower than beside the track. However, this is not the real damage. The damage had to be corrected for the real damaged surface by the tracks. This depends on the capacity (width) of the equipment used and the type of types used in practice. In this experiment the wheels of the equipment used will track on 25% of the total surface of a parcel ( $2 \times 0.65$  m / 5.20 m). Therefore, the real damage will be 0.25% of 17% = 4.25% of the DM yield without the machinery riding damage. This is the maximum estimated damage. In practice not the whole length of the track will be damaged, but only parts. There was no difference in damage between the plots 'Dry' and 'Wet'. That implies that probably on both plots the bearing capacity was under the threshold value for damage. Huijsmans et al. (1998) also observed this effect on clay. Under certain wet conditions, the bearing capacity was too low to prevent sward damage because the rolling resistance of the implement tyres increases and the traction potential of drive wheels decreases rapidly, both causing an increased risk of sward damage.

#### Conclusions

Increasing a low ditch level to shallow drainage reduces the bearing capacity under wet weather circumstances. The bearing capacity will recover slowly in spring. This means that slurry should be applied a few days later or, under very wet circumstances, even weeks later than at a low ditch level. Damage took place when slurry was spread at a bearing capacity of under 4 kg cm<sup>-2</sup>. The damage resulted in a 17% lower DM yield in the tracks. The real damage depends on the equipment used and the affected area.

## References

Beuving J., en Oostindië K. and Vellinga Th.V. (1989) Vertrappingsverliezen door onvoldoende draagkracht van veengrasland (Trampling losses by low bearing capacity on peat soil (summary in English)). Staring Centrum report 6, Wageningen.

Boxem Tj. and en Leusink A.W.F. (1978) Ontwatering van veengrasland. PR-publikatie ll.Lelystad (Drainage on peat grasslands in the Netherlands; summary in English).

Harville D.A. (1977) Maximum Likelihood approaches to Variance Component Estimation and to Related Problems. *Journal of American Statistical Association* 72, 358, 320–338.

Huijsmans J.F.M., Hendriks J.L.G. and Vermeulen G.D. (1998) Draught requirement of trailing foot and shallow injection equipment for applying slurry to grassland. *Journal of Agricultural Engineering Research* 71(4), 347–356

Schrier-Uijl A.P. (2010) Flushing meadows The influence of management alternatives on the greenhouse gas balance of fen meadow areas. *Thesis* Wageningen University.

## Changes in soil organic carbon in clay-loam soil following ploughing and reseeding of permanent grassland under moist temperate climatic conditions

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## Abstract

This study investigated (i) changes in soil organic carbon (SOC) content after ploughing and reseeding of permanent grassland (PG) over two and half years and (ii) changes of SOC under PG during seven years, on a poorly drained clay-loam soil at Solohead Research Farm (52°51'N, 08°21'W). In 2004, the swards were between ten and twenty years old. The experimental area was divided into four blocks with four paddocks per block of at least 1 ha. Bulked soil samples from PG paddocks per block were taken to 0.3 m depth on five occasions between 2004 and 2011. In 2008, one paddock per block was ploughed and reseeded and sampled as above on the last four occasions. SOC was determined by dry combustion. Carbon inputs to the soil organic matter and C leaching losses from PG and cultivated grassland (CG) were also investigated. Cultivation decreased SOC (P < 0.05) by 32.2 t ha<sup>-1</sup> in two and half years following ploughing. Simple linear regression indicated a tendency for PG to act as a C sink; however, the increase was not significant. High losses following cultivation had a significant impact on C balance of this ecosystem and therefore these results have potential implications for national greenhouse gas inventories.

Keywords: soil organic carbon, clay-loam soil, permanent grassland, cultivation

## Introduction

The International Panel on Climate Change fourth assessment identified agriculture as sector having the largest near term (by 2030) greenhouse gas (GHG) mitigation potential, largely via SOC sequestration. Signatories of the Kyoto protocol are committed to establish national inventories of SOC content. To date, national-scale inventories have not been able to clearly attribute changes in SOC to specific land use and management or climatic effects. The impact of renovation or reseeding of PG on SOC content and its potential contribution to carbon dioxide (CO<sub>2</sub>) emissions has yet to be quantified. The aim of this study was to investigate (i) changes in SOC content after ploughing and reseeding of PG over two and half years, and (ii) changes of SOC under PG during seven years, on a poorly drained clay-loam soil at Solohead Research Farm ( $52^{\circ}51^{\circ}N$ ,  $08^{\circ}21^{\circ}W$ ). In order to compare the levels of C inputs to the soil organic matter and C losses through leaching from PG and cultivated grassland (CG), aboveground phytomass productivity, root standing phytomass and dissolved organic carbon (DOC) leaching were also investigated.

#### Materials and methods

The farm has been under typical PG management for more than 50 years. In 2004, the swards were between ten and twenty years old. The experimental area (24 ha) was divided into four blocks with four paddocks per block of at least 1 ha. Bulked soil samples from PG in each block were taken to a depth of 0.3 m on each of five occasions between 2004 and 2011. In June 2008, one paddock per block was inversion ploughed to 0.2 m, reseeded (CG) and sampled as above on four occasions between 2008 and 2011. SOC was determined by dry combustion. Soil bulk density (BD) of PG and CG was measured in 2008. Root standing phytomass (0 to 0.3 m) was estimated by coring method in September 2010. At the same time, stubble phytomass (0 to 0.05 m) was harvested using scissors. The herbage phytomass was sampled repeatedly before grazing by cutting strips using lawn-mower. Aboveground phytomass productivity of PG and CG was obtained as a sum of pre-grazed herbage masses and stubble phytomass. DOC in shallow groundwater (0-2.2 m below ground level) under PG and CG was sampled between March 2009 and January 2010. Statistical analyses were performed using SAS, version 9.01 (SAS Institute Inc., Cary, North California, USA). A one-way analysis of variance was used to examine the differences in aboveground productivity, root standing phytomass, DOC annual losses and BD between PG and CG. A two-way analysis of variance was used to compare SOC between PG and CG over time while simple linear regression was used to estimate the annual change in SOC as a function of time.

#### Results

Grassland cultivation decreased SOC content (P < 0.05) in the upper 0.3 m of the soil profile (Figure 1). It had no effect on BD. There was no effect of sampling date on SOC under CG in two and half years following ploughing. The difference in SOC between PG and CG represented an average loss of 32.2 t C ha<sup>-1</sup> or, a reduction in topsoil SOC of 0.22 compared with PG. Most of the loss occurred during the first four months after cultivation.



Figure 1. Soil organic carbon (SOC) under PG and CG at 0 to 0.3 m depth. Error bars are  $\pm$  SE

The aboveground primary productivity, root standing phytomass and DOC annual losses were not significantly different between the grassland treatments (Table 1). The SOC content of the topsoil under PG displayed a considerable temporal variation (P < 0.05). The slope of linear regression line plotted for period 2004 and 2011 indicated tendency of SOC to increase, although this increase was not significant (y = 2.1318 x + 136.52,  $R^2 = 0.069$ , P = 0.214, where x was year and y was SOC (t ha<sup>-1</sup>)).

	Permanent grassland			Cultivated grassland				Statistic	
	Dry mass	SE	С	C:N	Dry mass	SE	С	C:N	Statistic
	Above	ground	l prima	ry produ	activity (t h	a <sup>−1</sup> ):			
Utilized phytomass	9.08	0.32	3.94	16.50	9.60	0.38	4.17	16.50	ns
> 0.05 m		0.70							
Stubble <0.05 m	2.16	0.53	0.87	18.63	2.63	0.33	1.01	20.70	ns
Belowground standing phytomass (t $ha^{-1}$ ):									
0-0.15	15.17	4.15	3.87	16.16	14.57	3.77	4.11	19.45	ns
0.15-0.30	0.82	0.19	0.29	25.70	0.33	0.10	0.12	29.59	ns
Roots 0-0.30	15.99		4.16		14.90		4.23		
DOC leaching (t ha <sup>-1</sup> ):			0.07	12.20			0.06	15.70	ns

Table 1. Aboveground and belowground phytomass and dissolved organic carbon losses to groundwater from permanent and cultivated grassland at Solohead Research Farm between 2008 and 2009

#### Discussion

As the aboveground primary productivity and root standing phytomass were not affected by the grassland cultivation, it is assumed that the C inputs into soil organic matter via decay of herbage residues and root material did not differ between the treatments. This indicates that the decrease in SOC content after grassland cultivation was mainly attributed to soil disturbance by ploughing rather than differences in C inputs to the soil. It is generally accepted that disruption of soil aggregates increases soil aeration, exposes stable, adsorbed soil organic matter to rapid oxidation and thus causes substantial ecosystem losses (Six et al., 2002). Since the DOC losses did not increase as a result of grassland cultivation during the study period, heterotrophic respiration was assumed to be the most important mechanism responsible for the C loss from the CG in this study. Consequently, cultivation resulted in a CO<sub>2</sub> flux of 101 t ha<sup>-1</sup> in the first four months between June 2008 and October 2008 and total CO<sub>2</sub> emissions of 118 t ha<sup>-1</sup> in two and half years following ploughing. Hence, approximately 0.86 of the mineralization took place in the first four months. The measured losses were several fold higher compared with other findings (Eriksen and Jensen, 2001). This can be attributed to the fact that Solohead PG is based on a heavy clay loam soil with high SOC content, which had been built up over many years under PG. Since there was no effect of sampling date on SOC under CG in a two and half years following ploughing, there was no evidence of accelerated recovery of SOC levels. Considering the uncertainty of the mean SOC resulting from its spatial variability, it is generally hardly possible to detect an increase in SOC within a few years. The statistical approach using simple linear regression indicated a tendency of PG to act as a C sink at rate of 2.1 C t ha<sup>-1</sup> yr<sup>-1</sup> between 2004 and 2011; however, the increase was not significant. In order to fully investigate the C accumulation on this site longer elapsed time research is needed.

#### Conclusions

The losses following cultivation had a significant impact on the C balance of this grassland ecosystem and therefore they have potential implications for national GHG inventories.

#### Acknowledgements

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#### References

Eriksen J. and Jensen L.S. (2001) Soil respiration, nitrogen mineralization and uptake in barley following cultivation of grazed grasslands. *Biology and Fertility of Soils* 33, 139–145.

Six J., Feller C., Denef K., Ogle S.M., Sa J.C.M. and Albrecht A. (2002) Soil organic matter, biota and aggregation in temperate and tropical soils – effects of no-tillage. *Agronomie* 22, 755–775.

## The effect of grassland management on enchytraeids (Oligochaeta) communities

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## Abstract

Enchytraeids (small white earthworms between 3 to 35 mm) are important regulators of nitrogen turnover in grasslands, as their activities accelerate the decomposition and nutrient recycling processes. In this study, the effect of management on species composition, abundance and biomass of the enchytraeid community was determined at three sampling occasions (before slurry application in October 2010 and March 2011, and after slurry application in May 2011) in 1-yr-old grass-clover field with three managements: 1) cut without manure, 2) cut with cattle slurry, and 3) grazed by heifers. We observed a significant effect of management on the enchytraeid biomass and density but no significant changes in their species composition. The slurry plot had the significantly highest biomass for the three management practices, in particular when compared to the grazed plots. We suggest that the lower enchytraeids biomass and density of the grazed plots are due to compaction by grazing animals.

Keyword: grass-clover, cattle manure, grazing, cutting, soil-fauna

#### Introduction

Grasslands cover 20% of the agricultural area in Denmark and are used for conventional and organic dairy farming. Dairy production systems are to a large extent based on these highly productive temporary grasslands. A range of soil organisms are involved in the decomposition processes and the nutrient mineralization in grassland and it has been reported that enchytraeids can be important regulators of nitrogen (N) turnover in native grasslands (Didden, 1993). Their activity stimulates soil microbial activity and affects soil structure by ingesting and excreting soil particles and creating burrows that cause increased soil aggregation and porosity (Didden, 1993; Bardgett, 2005). Despite the widespread abundance of enchytraeids in agricultural grasslands their responses to different agricultural management practices has largely been neglected. Grasslands in Denmark are dominated by grass-clover, and typical managements are the removal of herbage through grazing or cutting and application of fertilizer through injection of cattle slurry (Eriksen *et al.*, 2004). Those management practices are applied either alone or in combination and it is suspected that they can have different impacts on the enchytraeid communities.

Cutting grass can affect the below-ground soil food web by changing the carbon allocation from the roots as large amounts of organic matter are removed. Grazing is a combination of several factors that affect simultaneously the grassland ecosystem. Increased compaction can occur due to trampling, reduced input of organic matter due to grazing and a heterogenic input of nutrients and organic matter caused by the return of dung and urine (Bardgett, 2005). Soil compaction limits habitable pore space for soil fauna (Larsen *et al.*, 2004) and to our knowledge only a few studies have examined the effects on enchytraeids. Long term slurry application may have a positive effect on the enchytraeid community (King and Hutchinson, 1976) as it adds organic matter to the soil, which may result in increase food source for the enchytraeids. The objective of this study was to disentangle the effect on the enchytraeid community of different management practices applied to grassland used for dairy production.

## Materials and methods

The experimental plots were located in an arable-ley rotation experiment at Aarhus University, Foulum (9°34 E, 56°29 N); mean annual rainfall is 770 mm and mean annual temperature is 7.7°C. The grass-clover swards consisted of *Lolium perenne*, *Trifolium repens* and *Trifolium pratense*. The study presents investigations conducted with three managements: 1) cut without manure, 2) cut with cattle slurry (200 kg total N per ha), and 3) grazed by heifers (Table 1).

Table	1	Managements	111	experimental	orassland	nlots
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Abbreviation	Management	Fertilizer amendment		
CUT	Herbage removal	None		
CUT-SLURRY	Herbage removal	200 kg N <sup>1)</sup>		
GRAZED	Grazing <sup>2)</sup>	None <sup>3)</sup>		

<sup>1)</sup> Total-N in cattle slurry injected in April and June.

<sup>2)</sup> Grazed by 9 heifers per ha from May to October (1250 grazing days/ha/season).

<sup>3)</sup> Except for urine and dung from grazing cattle.

Samples were collected on 25 October 2010, 21 March 2011 and 2 May 2011. The second sampling occurred two weeks before slurry injection, and the third within the month following slurry application. At the first sampling date, three subsamples were collected from the CUT, GRAZED, and CUT-SLURRY treatments in one field block, and during the second and third samplings three subsamples were collected in each treatment in four replicated field plots. However, it was not possible to collect all the samples in GRAZED during the last sampling occasion due to soil compaction. The samplings were performed with a soil corer (inner diameter 5.5 cm; depth 18 cm) and the samples were kept at 5°C until extraction, which was initiated within two weeks. The total density of enchytraeids (per m<sup>2</sup>) and their biomass (dry mass per m<sup>2</sup>) was determined by integrating the 0–9 cm depth for all samples. The data were 'log+1' transformed and analysed as a randomized ANOVA repeated block design using the SAS PROC MIXED procedure.

## **Results and discussion**

The different management practices and dates of sampling both affected significantly the enchytraeid community in terms of abundance and biomass (Figure 1A and 1B). However, the species composition was not affected by management or season (data not shown). Both biomass and density varied with seasons (P < 0.001), with higher biomass and density of enchytraeids in all treatments in October 2010 compared to March and May 2011 (Figure 1A and 1B).

Enchytraeids are highly sensitive to drought and sub-zero temperatures (Maraldo and Holmstrup, 2009). Thus, the severe decrease in the enchytraeids community can be explained by the period with low soil moisture content between March 2011 and May 2011, where the soil water content decreased from 17% to 10%. The different management practices affected significantly the density (P = 0.013) and biomass (P = 0.004), with higher values in CUT plots compared to GRAZED but the effect varied between seasons. Both density and biomass were 3 to 5 times higher in CUT compared to GRAZED plots in October. Values were lower during the following spring (Figure 1) but this pattern was still in visible in March 2011, where CUT-SLURRY both had significantly higher enchytraeid biomass and density compared to GRAZED, and significantly higher density compared to CUT. However, the effect of the management was not significant in May 2011. The negative effect of grazing may be explained by the compaction of the soil by the heifers, as both biomass (P = 0.0083) and density (P = 0.0004) were lower in the 0–9 cm compared to the 9–18 cm layer (data not shown). It can be speculated that the density might be higher in hotspots with cow dung that typically have little or no trampling, but to avoid bias we did not sample in these locations. The injection of cattle slurry showed a stimulation of the enchytraeid community, which is due to the increase in organic matter inputs, but more studies involving older grassland is needed to conclude if the effect persists in the long-term.



Figure 1. (A) Total density (individuals per m<sup>2</sup>; mean  $\pm$  S.E; N = 2–4) and (B) total biomass (mg DW per m<sup>2</sup>; mean  $\pm$  S.E; N = 2–4) of enchytraeids according to grassland managements and season. Small letters show significant differences between managements within dates (GLM), and large letter shows significant between dates but within the same management practice (repeated ANOVA)

#### Conclusions

The different management practices clearly affected the enchytraeid community in different directions in 1-yr-old grass-clover field, with a positive effect of the density of cattle slurry addition and a negative effect by heifer compaction. However, more studies are needed to determine if the observed effect persists over time and hence to quantify the contribution of the enchytraeids to the decomposition processes in agricultural grasslands with different management practices.

#### References

Bardgett R.D. (2005) The biology of soil. Oxford University Press Inc., New York, US.

Didden W.A.M. (1993) Ecology of terrestrial Enchytraeidae. Pedobiologia 37, 2-29.

King K.L. and Hutchinson K.J. (1976) The effects of sheep stocking intensity on the abundance and distribution of mesofauna in pastures. *Journal of Applied Ecology* 13, 41–55.

Larsen T., Schjonning P. and Axelsen J. (2004) The impact of soil compaction on eucdaphic Collembola. *Applied Soil Ecology* 26, 273–281.

Maraldo K. and Holmstrup M. (2009) Recovery of enchytraeid populations after severe drought events. *Applied Soil Ecology* 42, 227–235.

# Abundance of azotobacter in the rhisosphere of alfalfa grown on different soil types

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## Abstract

In order to improve productive traits of soil and production potential of plants, microbial inoculation is increasingly being applied. In these studies, the effect of inoculation with rhizobium, azotobacter and actinomycetes, individually and in combinations, on the abundance of azotobacter in the rhizosphere of alfalfa grown on soils of different pH values was examined. Alfalfa seed was inoculated with the following inoculants: rhizobium *(Rhizobium meliloti)*, azotobacter (*Azotobacter chroococcum*) and actinomycetes (*Streptomyces* sp.). These microorganisms are useful as individual and combined cultures (a combination of two or all three types of microorganisms). The control variant was without inoculation. After inoculation, the seeds were sown in each variant in vessels filled with soil (semi-controlled conditions). After the first cutting of alfalfa, the number of azotobacter was determined in the rhizosphere of alfalfa in each treatment. The number of azotobacter was significantly different in the rhizosphere of alfalfa grown on each of the two soil types for all inoculation treatments when compared to the control variant.

Keywords: actinomycetes, alfalfa, azotobacter, rhisosphere, rhizobium, soil microbiology

## Introduction

Free nitrogen fixators, of which the most important are the bacteria of the genus *Azotobacter*, make an important contribution to providing nitrogen to plants and to increased nitrogen content of soil. Depending on the prevailing conditions in the soil, azotobacter may process  $50-80 \text{ kg ha}^{-1}$  of nitrogen from the air. Besides binding elemental nitrogen, azotobacter produces biologically active substances: auxin, gibberellins, pyridoxine, biotin and nicotinic acid (Dobbelaere *et al.*, 2003). Azotobacter reacts to minimal changes in soil conditions by reducing their numbers, which is why it can be used as an indicator of soil quality. Acidic reaction of the environment has a negative effect on the activity and abundance of azotobacter since these bacteria prefer a highly productive neutral soil. Present knowledge on the functioning of microbes and alfalfa symbiosis, and the possibilities of directing the microbial activities in order to increase the content of plant nutrients, indicate the need for application of microbial inoculants.

The aim of this study was to investigate the effect of rhizobium, azotobacter and actinomycetes, individually (monovalent inoculum) or in combination (polyvalent inoculum), on the number of azotobacter in the rhizosphere of alfalfa.

## Materials and methods

Two types of soil were used and they differed in their chemical characteristics. The soil samples were taken from two locations: Mačkovac (A) and Gaglovo (B) (Table 1). Soil A is of alluvium type, and soil B is a cambisol. Soil samples were taken to 30 cm depth. The inoculation was done using the different inoculums: 1. *Rhizobium meliloti*; 2. *Azo-tobacter chroococcum* 3. *Actinomycetes (Streptomyces* sp.); 4. *Rhizobium meliloti* +
Azotobacter chroococcum; 5. Rhizobium meliloti + Actinomycetes (Streptomyces sp.); 6. Azotobacter chroococcum + Actinomycetes (Streptomyces sp.); 7. Rhizobium meliloti + Azotobacter chroococcum + Actinomycetes (Streptomyces sp.); 8. Control – no inoculation. The varieties of Rhizobium meliloti (varieties L7 and L17) and Azotobacter chroococcum bacteria and the varieties of actinomycetes – Streptomyces sp. (varieties 5, 7 and 9K) were used. These microorganisms originated from the Department of Microbiology of the Faculty of Agriculture in Novi Sad.

	1				
Parameter	Total nitro-	$P_2O_5$	K <sub>2</sub> O	Humus	nH VCl
Soil	gen (%)	$(mg \ 100g^{-1})$	$(mg \ 100g^{-1})$	(%)	pri KCi
А	0.138	6.60	24.05	2.62	6.586
В	0.204	10.20	51.0	4.30	4.97

Table 1. The chemical composition of the soil

The genotype of alfalfa K-28 (Serbia) was used for this study. The alfalfa seed was sterilized using a 0.2% HgCl<sub>2</sub> solution and 70% alcohol rinsed several times with sterile tap water and afterwards submerged in the correct inoculum. After this, the seed of each inoculum variant was planted in pots of the two soils type. Ten millilitres of the corresponding inoculums were put in each pot. Two months after sowing, in the blossoming phase, the analysis of the epigeous part and the root of the plants were performed. Also at the same time, soil samples were taken for the microbiological analysis. The number of azotobacter was determined by the method of fertile drops of soil suspension 10<sup>-1</sup> on substrate by Fedorov. There were three replications for each sample. Statistical analysis was done using Costat computer program, and the significance of effects of applied treatments was determined using LSD test.

## **Results and discussion**

In our studies, in all inoculation treatments, as well as in the control variant, azotobacter number was higher in soil of neutral reaction (Figure 1). Acidic soil had unfavourable properties, poor in physiologically active nutrients, and an unsatisfactory air-water regime, so that the presence of azotobacter in these soils was very low or even non-existent (Miličić and Jarak, 2006). Due to the application of azotobacter its abundance increased in all inoculation treatments in which these bacteria were involved in both soil types. A significant positive impact of applied measures was noted in the soil of increased acidity as the number of microorganisms which will survive after the introduction into the soil depends to a great extent on the conditions prevailing in it.



Figure 1. The number of azotobacter (log of number) in the rhizosphere of alfalfa grown on different soil types

#### Conclusions

Application of the inoculation of alfalfa seed using selected strains of azotobacter, whether as a monovalent or combined with actinomycetes and rhizobium (polyvalent inoculum), led to an increase in the number of the tested micro-organisms. This is especially important for soil with increased acidity, and the intensification of microbiological processes is due to the adaptation of those micro-organisms to the requirements of the new environment. In the comparisons of the abundance of azotobacter in samples of the studied soils, in all inoculation treatments the azotobacter number was significantly higher in soil of neutral reaction.

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#### References

Dobbelaere S., Vanderleyden J. and Yaacov O. (2003) Plant growth promotingeffect of diazothrophs in the rhizosphere. *Critical Reviews in Plant Science* 22, 107–148.

Miličić D. and Jarak M. (2006) Microbiological activity in soil with different acidity. Biotechnology 2006, *Scientific Pedagogical Publishing*, Č. *Budejovice*, *Czech Republic*.

# Indicating soil quality using urease and saccharase activity in abandoned grassland and differently managed crop fields

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## Abstract

In order to ascertain and make a comparison of bioactivity variation during the vegetation period, soil (*Hapli–Epihypogleyic Luvisol* (*LVg-p-w-ha*), *Albi–Epihypogleyic Luvisol* (*LVg-p-w-ab*), and *Hapli–Albic Luvisol* (*LVA-Ha*), artificial drainage) samples were collected in fields of different fertilization and farming systems, namely extensive (ExF), conventional (CF) and organic (OF), at the Lithuanian University of Agriculture during 2007–2009. The aim of this study was to determine the soil bioactivity responses (saccharase and urease activity) to different land management systems and main soil quality indices. The trial comprised eight experimental plots (three land management systems and two crop groups: legumes and grasses).

Assessed hydrolases were sensitive indicators suitable to evaluate parameters of soil quality. The highest decrease and the lowest rates of urease (1.13 mg  $NH_4^+-N$  g<sup>-1</sup>) and saccharase (8.40 mg CG g<sup>-1</sup> 24 h<sup>-1</sup>) activities were observed in abandoned grassland soil where mineral fertilizers were not applied. Enzyme activity was more strongly correlated with SOC (r = 0.7) or C/N ratio (r = 0.6) than that with total nitrogen content (r = 0.5-0.6). Significantly higher 3-year mean values of saccharase (27.00 and 12.6 mg of conventional glucose, CG g<sup>-1</sup>) and urease (5.78 and 4.16 mg  $NH_4^+-N$  g<sup>-1</sup> 24 h<sup>-1</sup>) were observed in soil under conventional and organic management in comparison with abandoned grassland. Consequently, it can be concluded that enzyme activities might be responsible for the different levels of soil fertility.

Keywords: agro-ecology, indicators, enzyme activity, farming management, crop

# Introduction

Soil fertility plays an important role for the sustainable development of terrestrial agroecosystems and depends mainly on results of organic matter decomposition to compounds of low molecular weight and dissolved organic compounds. Soil biota govern the soil role in metabolism processes of materials and energy in ecosystems and represent an integrated index of soil physical-chemical conditions and soil quality (Dilly et al., 2007). Moreover, soil biodiversity is referred to as conferring stability to stress and disturbance, but the mechanism is not yet fully understood. Dissolved organic matter is produced by soil enzyme-catalysed depolymerization of organic matter and is comprised of low molecular weight compounds that are often water soluble and thus more accessible to biota assimilation as energy, carbon, and nutrient sources. Thus, soil microorganisms and enzymes are the primary mediators of soil biological processes, including organic matter degradation, mineralization and nutrient recycling (Li et al., 2008). Hydrolytic enzymes are influenced by a wide range of soil properties such as pH, organic matter and texture, and also by farming management and anthropogenic impacts. Saccharase and urease are related to the C and N cycles, which are the fundamental factors in forming soil fertility. Among other indices, enzyme activity is proposed as an index of soil fertility or contamination (Li et al., 2008).

The main aim of this study was to evaluate the impact of farming management system (conventional, organic and extensive) and different crops (legumes and grasses) on activity of urease and saccharase related to the C and N cycles in the short term on different

soils. Moreover, discrimination of soil hydrolases properties in organic and conventional management systems was determined.

#### Materials and methods

Soil samples were collected in accordance with ISO 10384–1:2002 from different farming fields at 8 sites: extensive (ExF: abandoned barley-couch grass (b-cg; *Hapli–Epihypogleyic Luvisol, LVg-p-w-ha*;  $N_{120}P_{50}K_{60-0}$ ), abandoned meadow (g; *Hapli–Albic Luvisol, LVA-Ha*) and fodder galega (*Galega orientalis* Lam.) stand (lga; *Hapli–Albic Luvisol, LVA-Ha*); conventional (CF: 1) oat-vetch-winter wheat-barley-clover, ov-w-bcl; *Hapli–Epihypogleyic Luvisol, LVg-p-w-ha*;  $N_{60}P_{50}K_{60}$ - $N_{120}P_{50}K_{60}$ ,  $N_{60}P_{50}K_{60}$ ; 2) oat-vetch-barley, ov-b; *Albi–Epihypogleyic Luvisol, LVg-p-w-ab*;  $N_{120}P_{50}K_{60}$ , and 3) w. wheat-oilseed rape-bare fallow, w-r-bf; *Albi–Epihypogleyic Luvisol, LVg-p-w-ab*;  $N_{120}P_{50}K_{60}$ , and 3) w. orp-b; *Hapli–Epihypogleyic Luvisol, LVg-p-w-ab*;  $N_{120}P_{50}K_{60}$ - $N_{120}P_{50}K_{60-0}$ ), and organic (OF: oat-pea-barley-barley-clover, op-b-bc and w.wheat-oat-pea-barley, w-op-b; *Hapli–Epihypogleyic Luvisol, LVg-p-w-ha*; manure, 80 t ha<sup>-1</sup> yr<sup>-1</sup>) rotation crops in June of 2007–2009. OF is certificated by the EKOAGROS (Lithuanian Committee for Organic Agriculture). Weed control by tillage and herbicide was applied only in agri-phytocoenoses of conventional farming. Soil samples were taken in triplicate. Soil pH was recorded potentiometrically using 1 n KCl extraction, mobile  $P_2O_5$  and  $K_2O$  (mg kg<sup>-1</sup> of soil) by A–L method. This study relies on analysis data obtained within a joint project presented by Sabiene *et al.* (2010).

Soil bioactivity was characterized by applying a bioassay of hydrolytic enzyme activity. The enzymatic activity was determined in air-dried soil samples. All treatments and measurements were replicated three times. Saccharase (EC 3.2.1.26) activity was measured according to the modified Hofmann and Seegerer method (Dilly *et al.*, 2007). Urease (EC 3.5.1.5) activity was assayed by employing Hofmann and Schmidt spectrometric method. Urease and saccharase activities were expressed as production rates of NH<sup>4+</sup>–N g<sup>-1</sup> or glucose g<sup>-1</sup>air dried soil (mg g<sup>-1</sup>).

## **Results and discussion**

Farming type and crop rotation indicated significant impact on soil fertility indices). Saccharase activity, with the exception of CF crop (r = 0.3) stands was observed to be significantly different and dependent on farming type as well as crop during the 3 study years (Figure 1). Conventional farming induced the highest saccharase activity in a winter wheat stand  $(27.00 \text{ mg g}^{-1})$  supposedly due to glycoside presence in root secretions (Groenigen et al., 2010), appropriate aeration conditions and sufficient C-containing substrate supply. Saccharase activity in CF fallow (17.18 mg  $g^{-1}$ ) and CF mixtures with legumes (15.87 and 14.95 mg  $g^{-1}$  respectively) was lower than in the CF w. wheat treatment. Extensive farming did not stimulate indices of soil fertility (SOC, N accumulation and C/N rise) and saccharase activity. Response of saccharase activity to different farming types could be explained by its strong correlation with SOC content (r = 0.7), which varied across different farming systems and ranged between 4.86 in ExF meadow and 12.4% in IF w. wheat stands. The highest mean values of soil fertility indices were observed in fields under the conventional (17 g kg<sup>-1</sup> SOC, 1.4g kg<sup>-1</sup> N and 12 C/N ratio) and organic (17.5 g kg<sup>-1</sup> SOC, 1.36 g kg<sup>-1</sup> N and 13 C/N ratio) management system. In accordance with chemical parameters of soil fertility, the highest mean activity of saccharase was observed in CF (19.71 mg CG  $g^{-1}$ ) and OF (12.57 mg  $g^{-1}$ ) respectively. Therefore our study demonstrated that conventionally and organically managed soils exhibited greater saccharase activity due to beneficial conditions for an accumulation of SOC (r = 0.7) and a optimal C/N ratio (r = 0.7), which, in turn, guarantee a sufficient amount of C-containing substrate. Uncultivated and unfertilized soils of ExF did not accumulate organic matter. Consequently, the lowest SOC content (5.67-4.86 g kg<sup>-1</sup>) and saccharase activity (8.6–11 mg  $g^{-1}$ ) were observed in ExF.



Figure 1. Saccharase and urease activity responses to farming type and different crop stands (mean $\pm$  SE, P < 0.05)

Urease activity represents the potential nitrification rate and has attracted a considerable attention due to increasing application of urea as fertilizer to improve the soil fertility. Similarly, as in the case of saccharase, different soil-urease activities were observed for different farming and crop types. Urease activity was related with nitrogen-containing substrates dynamics in response to land management intensity and crop (r = 0.6). The highest urease mean activity was observed in CF and OF wheat stands (5.79 and 4.16 mg g<sup>-1</sup>) possibly due to heavy nitrogen fertilization (N<sub>100-120</sub>).

High annual inputs are targeted to provide high crop yields. Nonetheless, the rise of urease activity was more strongly correlated with SOC (r = 0.7) or C/N ratio (r = 0.7) than with total nitrogen content (r = 0.6). A low urease activity was observed in soil of EF abandoned grassland (1.13 mg g<sup>-1</sup>) and barley-couch grass (1.67 mg g<sup>-1</sup>) due to their low total N (1.2 g kg<sup>-1</sup>) and SOC (11 g kg<sup>-1</sup>) content. Due to lower rates of soil quality indices, the lowest urease mean activity (2.27 mg g<sup>-1</sup>) was observed in fields under extensive farming. An increase of soil fertility indices in conventional (17 g kg<sup>-1</sup> SOC, 1.40 g kg<sup>-1</sup> N and 12.7 C/N ratio) and organically (17.5 g kg<sup>-1</sup> SOC, 1.36 g kg<sup>-1</sup> N and 13 C/N ratio) farmed soils indicated better agronomic management there. Land management stimulated the increase of mean urease activity in conventional (3.93 mg g<sup>-1</sup>) and organically (3.88 mg g<sup>-1</sup>) farmed soils.

#### Conclusions

Conventional and organical farming promoted the highest enzyme activities. Fluctuations in enzyme activities in experimental soil might have occurred due to different agronomic practices and soil fertility conditions during the crop seasons. More intensive studies are required to evaluate the effects of different farming practices on important soil enzymes important for soil health and soil fertility. Significant differences between observed farming management types and soil biochemical indices revealed the relevance of enzyme activity as a proxy indicator for evaluating soil fertility.

#### References

Dilly O., Munch J.Ch. and Pfeiffer E.M. (2007) Enzyme activities and litter decomposition in agricultural soils in northern, central, and southern Germany. *Journal of Plant Nutrient and Soil* 170, 197–204.

Li J., Zhao B., Li X., Jiang R. and Bing H.S. (2008) Effects of long-term combined application of organic and mineral fertilizers on microbial biomass, soil enzyme activities and soil fertility. *Agriculture Science in China* 7, 336–343.

Sabiene N., Kusliene G. and Zaleckas E. (2010) The influence of land use on soil organic carbon and nitrogen content and redox potential. Žemdirbystė- Agriculture 97, 15–24.

# The balances of Mg and its concentration in soil and ground water in conditions of diversified soil fertilization on the permanent peat meadow

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## Abstract

Production and environmental effects of fertilization methods were compared for a permanent meadow on a peat-muck soil (formed by drainage of peat). The following mineral fertilization methods were used: PK (P-30, K-60 kg ha<sup>-1</sup>), NPK at two levels: (N-60, P-30, K-60 kg ha<sup>-1</sup>) and (N-90, P-30, K-90 kg ha<sup>-1</sup>). Fertilization by manure and slurry was also used with two comparable levels. About 50 kg ha<sup>-1</sup> of Mg was introduced annually with manure, causing more positive balances, and about 15 kg ha<sup>-1</sup> of Mg was introduced annually with slurry, causing improvement of Mg balance in comparison with mineral fertilization. Fertilization with natural fertilizers increased the amount of Mg in the upper soil layers without increasing its concentration in ground water. Mg concentrations in water were determined by soil abundance.

Keywords: Mg in soil and ground water, different methods of fertilization, Mg balances

# Introduction

Natural fertilizers are a source of many important nutrients that affect yield and quality. One of the components significantly influencing feed quality and animal health is Mg (Marcinkowski, 1990). Mg concentration in the soil and vegetation is largely dependent on soil abundance of Mg (Gotkiewicz and Gotkiewicz, 1986) and its supply through fertilization. As indicated by Kuszelewski and Łabętowicz (1987), manure fertilization with other nutrients especially increases Mg abundance in Mg-deficient soils. The increasing abundance in Mg of upper layers of soil may be caused by its movement to the deeper layers (Fotyma and Gosek, 2001) and, as indicated by Łabętowicz and Rutkowska (2001), to ground water. The aim of this study was the calculation of Mg balances and identification of Mg economy.

## Materials and methods

The study was conducted in 2006–2008 on a permanent meadow in a field experiment on a peat-muck soil (formed by drainage of peat). Each field of surface area 0.3 ha was fertilized with mineral or natural fertilizers. The following mineral fertilization treatments were used: PK (P-30, K-60 kg ha<sup>-1</sup>), NPK at the first level (N-60, P-30, K-60 kg ha<sup>-1</sup>) and NPK at the second level (N-90, P-30, K-90 kg ha-1). Digested cattle manure and liquid manure were also used to supply the same doses as for the NPK fertilizer treatments. Manure was applied every year in autumn. Liquid manure was applied in spring and after the first cut. Doses of manure and liquid manure were determined on the basis of nitrogen content, including adopted equivalents of nitrogen, phosphorus and potassium utilization (from manure: N 0.5, P 1, K 0.7, and from liquid manure: N 0.7, P 1, K 0.8). Soil abundance of nutrients was determined before establishment of the experiment. The field experiment was cut three times per year. Dry matter yields and content of macronutrients in the meadow sward were determined. Yields from each field were determined in five fixed points (replicates). The Mg balances were calculated based on the Mg input with fertilizers and rainfall, and outputs with yields including the Mg content in the sward. The nutrient content in the groundwater from every field was examined four times per season.

## Results

Mg balances for individual treatments showed significant differences (Table 1), mainly due to the amount of Mg input. On the treatments fertilized with mineral fertilizers the only source was in rainfall. In this case the Mg input amounted 1.5 kg Mg ha<sup>-1</sup>. On the treatments fertilized with natural fertilizers, where the main source of Mg was from the fertilizers, the amount of Mg input ranged from about 7 to over 45 kg Mg ha<sup>-1</sup> each year. The Mg output in the yields showed much lower variation between individual treatments, ranging from about 16 to 31 kg Mg ha<sup>-1</sup> during in the period of comparisons. The Mg balances on all treatments fertilized with mineral fertilizers showed significantly negative values: from -15 to -30 kg of Mg ha<sup>-1</sup> with a trend towards increases in the following years. The balances of Mg on the treatments fertilized with manure were positive. For the first level of fertilization (M/I) they showed a high downward trend in the following years. For the second level (M/II) a significantly higher values of balances and higher alignment in the compared years were found. Treatments fertilized with liquid manure, at levels similar to those fertilized with mineral fertilizers, showed negative balances but they were much lower. Fertilization with higher level of liquid manure (LM/II) significantly reduced the negative balances of Mg in all years.

Years	Balance components	PK	NPK/I	NPK/II	M/I	M/II	LM/I	LM/II
	input (rainfall + fertilization)	1.5	1.5	1.5	28.7	42.3	7.2	10,0
2006	output	16.7	18.7	29.0	18.1	19.3	21.2	16.5
	balance	-15.2	-17.2	-27.5	10.6	23.0	-14.0	-6.5
2007	input (rainfall + fertilization)	1.5	1.5	1.5	31.1	45.8	8.0	10.2
	output	22.1	25.3	23.1	24.4	22.4	28.1	29.7
	balance	-20.6	-23.8	-21.6	6.7	23.4	-20.1	-19.5
2008	input (rainfall + fertilization)	1.5	1.5	1.5	28.5	42.1	7.8	9.9
	output	29.6	22.8	31.2	28.3	25.2	28.7	24.6
	balance	-28.1	-21.3	-29.7	0.2	16.9	-20.9	-14.7

Table 1. The balances of Mg on objects of differential fertilization (kg ha<sup>-1</sup>)

The soil abundance in available forms of Mg (Figure 1a) in the majority of experimental fields in 2006 in the 0–10 cm layer was in the range of its optimal value. High soil abundance in this layer was found mainly on treatments PK, NPK/I and M/I. In the 10–20 cm soil layer (Figure 1b) the abundance in Mg in all treatments was lower and only the NPK/I treatment was high. Three years fertilization with phosphorus and potassium (PK) and nitrogen fertilization at the first level (NPK/I) resulted in a reduction of soil abundance in Mg. The second level of NPK fertilization resulted in a slight growth. There was an increase of soil abundance in Mg in the 0–10 cm layer on all treatments fertilized with manure. There was a significant increase in the abundance of Mg in the 10–20 cm soil layer on all treatments, which indicates its movement in the soil.

The lowest concentration of Mg in ground water (Figure 2) as well as the soil abundance was found on the NPK/II treatment, and slightly higher values in 2007 and 2008 were found on the PK treatment. The ground waters during the study period were classified as first-class quality in both cases. The largest concentration of Mg in ground water, included within the second-class quality and slightly changing during the study, was found for the two treatments NPK/I and M/I (with the highest soil abundance in this component). The Mg concentrations in water classified as mainly of first-class quality, or on the border of the first and the second class, and changing slightly during the study period, were found on the following treatments: M/II, LM/I and LM/II. The Mg concentrations in ground



Figure 1. The soil abundance in available forms of Mg marked in 0.5 m HCl (in g kg<sup>-1</sup>)

water from these treatments showed significant differentiation mainly due to the soil abundance in this component.



#### Conclusions

Fertilization with natural fertilizers for improving the balance of Mg resulted in significantly increased Mg soil abundance. The increase of soil abundance in Mg in both the 0–10 and 10–20 cm layers, and for all treatments, indicates an ongoing process of its movement. The level of Mg of concentrations in the ground water on particular treatments shows that, despite of the high increase of Mg soil abundance in the upper layers of soil on the treatments fertilized with natural fertilizers, this did not cause deterioration of ground water quality.

## References

Fotyma M. and Gosek S. (2001) The movement of potassium and magnesium in the soil profile and fertilized with liquid manure with mineral fertilizers. *Advances in Agricultural Science Problem Issues* 480, 395–402 (in Polish).

Gotkiewicz J. and Gotkiewicz M. (1986) Effect of differentiated fertilization on soil, peat meadows on the amount and quality of the hay crop. *Symposium Proceedings* I. 2. Olsztyn (in Polish).

Kuszelewski L. and Łabętowicz J. (1992) Effect of fertilization with different nutrient diversity and sustainable use of manure on the yield of crops in rotation. P. I. Permanent fertilizer experience in Łyczyn (1960–1985). *Annals of Agricultural Science* A, 109, 3, 81–9 (in Polish).

Łabętowicz J. and Rutkowska B. (2001) Factors affecting the concentration of magnesium in the soil solution of soils used for agriculture in Poland. *Advances in Agricultural Science Problem Issues* 480, 103–111 (in Polish).

Marcinkowski T. (1990) Influence of soil and vegetation in the grassland at the level of magnesium in the blood serum of cattle on the example of Lomza province. PhD thesis. IMUZ Falenty (in Polish).

# A study of ingrowth-core sampling frequency in a managed European grassland

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#### Abstract

The ingrowth-core method (root diameter  $\leq 1.0$  mm, 0~30 cm vertical depth) was performed in a ryegrass (*Lolium perenne* L.) / white clover (*Trifolium repens* L.) mixture in North Germany in 2010 to investigate the belowground net primary production (BNPP) and specific root length (SRL) yielded from different sampling frequencies. From April to October the measured accumulated BNPP was 619, 609, 238 g m<sup>-2</sup> respectively and average SRL was 179, 248 and 235 m g<sup>-1</sup> respectively from 3 sets of ingrowth-core that had different ingrowth periods. Two sampling strategies were suggested from our results: continuously sampling at 6-week intervals could properly represent BNPP and SRL at this site, whilst a 3-week ingrowth interval could properly represent BNPP and fine root dynamics.

Keywords: fine root production, specific root length, ingrowth-core, grassland

#### Introduction

Fine root dynamics in European managed grasslands is far from clear, as most belowground net primary production (BNPP) studies have been applied to agricultural crops and forests. Meanwhile, the widely applied sequential core calculations for BNPP are controversial for the risk of either overestimation or underestimation. Therefore, in this study we aimed to investigate fine root (<1 mm) production and specific root length by means of the ingrowth-core method (Steingrobe, 2001) assuming that higher sampling frequency will induce higher BNPP and lower SRL.

## Materials and methods

The field experiment was conducted at Kiel University's experiment station Lindhof ( $53^{\circ}40'$  N;  $10^{\circ}35'$  E). The climate is temperate oceanic, with a mean annual temperature of  $8.7^{\circ}$ C and mean annual precipitation of 785 mm, and the main soil type is sandy loam with a pH 5.9. Swards were dominated by perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) The sward was cut four times at the height of 5 cm per year. Aiming at different time spans conducted in parallel (Figure 1), three sets of ingrowth-core were applied: the short-term ingrowth-core ('LT'). For each ST trial, 4 cores were installed in each plot at a distance of 30 cm; for each MT and LT trial, only 3 cores were installed as limited by labour. The installation of ingrowth-core is described in reported literature (Steingrobe, 2001). By each sampling date, cores were taken out and washed under tap water on a 0.63 mm sieve, and 2 g of fresh material was cut into 5 mm pieces for determining root length on a 1×1 cm grid using the line intersection method. Dry mass was recorded after drying at 58°C for 48 hours.

For the reason of comparability, when BNPP and SRL from different ingrowth-core sets were compared, we made a "stage comparison" as the following pairs showed:  $BNPP_{MT1}$  and  $(BNPP_{ST1} + BNPP_{ST2})$ ;  $BNPP_{MT2}$  and  $(BNPP_{ST3} + BNPP_{ST4})$ ;  $BNPP_{MT3}$  and  $(BNPP_{ST5} + BNPP_{ST4})$ ;  $BNPP_{MT3}$  and  $(BNPP_{ST5} + BNPP_{ST4})$ ;  $BNPP_{MT3}$  and  $(BNPP_{ST5} + BNPP_{ST5})$ ;  $BNPP_{ST5}$  and  $(BNPP_{ST5} + BNPP_{ST5})$  and (BN

BNPP<sub>ST6</sub>); BNPP<sub>MT4</sub> and (BNPP<sub>ST7</sub> + BNPP<sub>ST8</sub>). SRL<sub>MT1</sub> and (SRL<sub>ST1</sub> + SRL<sub>ST2</sub>)/2; SRL<sub>MT2</sub> and  $(\text{SRL}_{\text{ST3}} + \text{SRL}_{\text{ST4}})/2$ ;  $\text{SRL}_{\text{MT3}}$  and  $(\text{SRL}_{\text{ST5}} + \text{SRL}_{\text{ST6}})/2$ ;  $\text{SRL}_{\text{MT4}}$  and  $(\text{SRL}_{\text{ST7}} + \text{SRL}_{\text{ST8}})/2$ . For the annual BNPP and SRL, ANOVA was performed in the Mixed Model of SAS 9.1(SAS Institute). Among different sampling method, BNPP and SRL data in stage comparison were compared using paired t-tests. The P-values were adjusted by the Bonferroni-Holm procedure. All comparisons of least square means were based on an experimental error of  $\alpha = 0.05$ .

#### **Results and discussion**

The accumulated BNPP during the growing season from ST (BNPP<sub>st</sub>), MT (BNPP<sub>MT</sub>) and LT (BNPP<sub>1T</sub>) was 620, 582 and 227 g m<sup>-2</sup> respectively. BNPP<sub>ST</sub> and BNPP<sub>MT</sub> did not show significant difference, and both were significantly (P < 0.05) higher than BNPP<sub>1T</sub>. Only in the first stage (until late May) BNPP in ST was significantly (P < 0.001) lower than corresponding MT; the other 3 pairs showed no significant differences (Figure 2A). In terms of seasonal pattern, the BNPP curve resulting from each individual ST turned out to be bimodal. BNPP<sub>ST</sub> and BNPP<sub>MT</sub> data did not show significant difference; both were in a similar level to a former study in grassland using either ingrowth-core or minirhizotron (Steinaker et al., 2008; Garcia-Pausas et al., 2011). Assuming the differences between BNPP<sub>IT</sub> and BNPP<sub>ST</sub> BNPP<sub>MT</sub> were mainly contributed by fine root decomposition, the percentage of mass loss fell into the interval of the previous reported fine root decomposition rate among different climate zones (Fan et al., 2010). The seasonal pattern was also in accordance with a previous study using minirhizotron (Steinaker et al., 2008). The absolute BNPP yield, mass loss percentage and seasonal pattern together suggested that the application of ST and MT ingrowth-core on BNPP study in this site performed rationally. Moreover, ST could represent the seasonal change of fine root dynamics.



egy of 3 sets ingrowth-core

The accordance between ST and MT pairs (except the first stage), together with the accumulated BNPP results, indicated: 1) that the effect of installation (either positive or negative by injury and modified environment) did not differ between ST and MT; 2) that the fine root decomposition in one month was not substantial. The marked difference in spring between ST and MT might be caused by the low temperature and installation together. Average SRL from ST (SRL<sub>ST</sub>), MT (SRL<sub>MT</sub>) and LT (SRL<sub>1T</sub>) were 179, 248 and 235 m g<sup>-1</sup> respectively.  $SRL_{MT}$  and  $SRL_{LT}$  were not significantly different; both of them were significantly higher than SRL<sub>sr</sub> (P < 0.05). Interestingly, the first three stages of SRL from ST were all significantly lower than corresponding MT (P < 0.05) but the last pair of ST and MT, which had a longer duration (in this case, an individual ST covered for 4~5 weeks), showed no difference (Figure 2B).

Our results indicated that the development of a complete fine-root system in this grassland needs more than 3 weeks' time. As a former study showed this fine root cohort (diameter <1 mm) included at least 4 root orders of fine roots from a minirizotron study (Guo et al., 2004), the relative higher SRL<sub>MT</sub> and SRL<sub>IT</sub> might be caused by development of both finer roots



Figure 2. BNPP (A) and SRL (B) from ST and MT. Columns show stage comparison, and curve show individual BNPP (A) and SRL (B) from ST. The asterisk indicates significant difference between ST and MT at  $\alpha$ -level of 0.05

and/or lower order roots after 3 weeks' ingrowth period. Moreover, the statistically same level of  $SRL_{MT}$  and  $SRL_{LT}$  also indicated in this study the well-developed root system might reached its potential level and the SRL was confined by both intrinsic characteristics and site conditions.

#### Conclusions

In this work we investigated the belowground net primary production (BNPP) and specific root length (SRL) by means of the ingrowth-core method in a ryegrass-white clover sward in North Germany in 2010. Our results showed that reliable BNPP and SRL data could be represented by this method. It is worth noting that the sampling frequency should be adjusted according to different root growth rate affected by site conditions if this method is to be applied to other grassland sites.

#### References

Fan P.P. and Guo D.L. (2010) Slow decomposition of lower order roots: a key mechanism of root carbon and nutrient retention in the soil. *Oecologia* 163, 509–515.

Garcia-Pausas J., Casals P., Romanyà J., Vallecillo S. and Sebastià M.-T. (2011) Seasonal patterns of BGB and productivity in mountain grasslands in the Pyrenees. *Plant and Soil* 340, 315–326.

Guo D.L, Mitchell R.J. and Hendricks J.J. (2004) Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. *Oecologia* 140, 450–457.

Steinaker D.F. and Wilson S.D. (2008) Phenology of fine roots and leaves in forest and grassland. *Journal of Ecology* 96, 1222–1229.

Steingrobe B., Schmid H., Guster R. and Claassen N. (2001) Root production and root mortality of winter wheat grown on sandy and loamy soils in different farming systems. *Biol Fertil Soils* 33, 331–339.

# Greenhouse gas balance of bioenergy cropping systems under the environmental conditions of Schleswig-Holstein

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#### Abstract

A 2-year field trial was conducted at two sites in Schleswig-Holstein, northern Germany, to perform a GHG balance to quantify and evaluate the impact of cropping system (maize monoculture, maize-wheat rotation and permanent grassland), nitrogen (N) level and N type. Considering the whole production chain, maize monoculture revealed a higher GHG mitigation potential than the other systems investigated. The comparison of fertiliser types showed less pronounced differences in GHG emission, whereas considerable site effects were observed.

Keywords: GHG balance, biogas, maize, permanent grassland

#### Introduction

In Germany, biogas from energy crops and slurry is being promoted as an efficient means of contributing to a reduction of GHG emissions compared with fossil sources. There has been a considerable expansion of biogas plants in the federal state of Schleswig-Holstein, northern Germany, with 500 plants established by mid-2011. Due to the resulting expansion of maize production, criticism on the GHG mitigation potential of biogas production has been voiced recently. Although various life cycle assessments (LCA) of biogas production are available, many of these are based only on literature data, especially with regard to energy and GHG balances. Furthermore, data for northern Germany are generally limited. Therefore the aim of this study was to generate a GHG balance for biogas production systems in northern Germany and to evaluate the GHG mitigation potential based on a 2-year field trial.

#### Materials and methods

Within the framework of the joint project Biogas-Expert of Kiel University, a 2-year field trial (2007–2009) was conducted at two experimental sites, Hohenschulen (HS) and Karkendamm (KD), in northern Germany. The overall objective of the project was to contribute to a sustainable optimisation of biogas production by comprehensive experimental trials and systems modelling. The annual precipitation at the experimental site HS averages 750 mm and mean daily temperature is about 8.3°C. The soil is classified as a pseudogleyic Luvisol of sandy loam structure. The annual precipitation at the station KD averages 844 mm with a mean daily temperature of about 8.3°C. The soil is classified as a gleyic Pod-zol of sandy structure. Altogether, three cropping systems have been investigated: maize monoculture and a maize–whole crop wheat–Italian ryegrass rotation at HS, while maize monoculture and a four-cut permanent grassland were tested at KD. N-fertiliser was applied at four levels (maize, wheat: 0, 120, 240, 360 kg N ha<sup>-1</sup>; grassland: 0, 160, 320, 480 kg N ha<sup>-1</sup>) and different N types: calcium ammonium nitrate (CAN) and biogas residue from co-fermentation was applied at KD and HS, with cattle slurry additionally applied at KD and pig slurry at HS.

GHG balances were calculated according to the life cycle inventory analysis provided by the ISO guidelines 14044 (2006). The calculations are based on the assumptions and results of the energy balance by Claus *et al.* (2010) supplemented by measured data of  $N_2O$  emissions (Senbayram et al., 2009). Measurements of NH<sub>3</sub> emissions (Gericke, 2009) were taken into account to estimate the indirect N<sub>2</sub>O emissions. The increase and decrease in soil carbon stocks have been considered according to German cross-compliance commitments. Emissions related to plant cultivation, transport, storage of energy crops and the conversion in a biogas plant were allocated to the biogas production process. Since slurry is considered to be a waste product from livestock production, only emissions from slurry storage (90 days) were accounted for. For conversion, a combined heat and power plant (500 kW), with an electric efficiency of 40%, a thermal efficiency of 41.5% and heat utilization of 45% was assumed. Heat and electricity demand for plant operation were assumed to be 20% of the generated electricity for heat and 7.5% for electricity. The relation of N input to total emissions of CO<sub>2</sub>eq. resulting from electricity and heat generation was fitted by assuming an exponential function. GHG emissions were compared with a reference system, in which electricity is produced by using fossil sources (0.72)kg CO<sub>2</sub>eq. kWh<sub>el</sub><sup>-1</sup>) and heat by using heating oil (0.31 kg CO<sub>2</sub>eq. kWh<sub>th</sub><sup>-1</sup>). The GHG saving potential was obtained by substracting the emissions for producing electricity and heat by biogas from the emissions of the reference system.

#### **Results and discussion**

The comparison of cropping systems at HS revealed noticeably higher GHG emissions for the production of energy and heat by biogas from maize monoculture than from the maize–whole crop wheat–Italian ryegrass rotation (Figure 1a). In agreement, higher total GHG emissions were found for maize monoculture than for permanent grassland at KD (Figure 1b). The higher emissions were caused by much higher  $N_2O$  fluxes during maize cultivation (Senbayram *et al.*, 2009). Furthermore, maize achieved higher biomass yields at both sites, which caused a higher heat and electricity demand for the conversion to biogas. Since the GHG saving potential was obtained by subtracting the emissions for producing electricity and heat by biogas from the emissions of the reference system, the higher GHG emissions in turn did not result in a lower GHG saving potential (Figure 1a, 1b). This is because the higher yields of maize mono-culture resulted in higher net energy yields. Therefore maize



Figure 1. Relationship between total N-input (kg N ha<sup>-1</sup>),  $CO_2$ eq. emissions for the production of energy and heat by biogas and  $CO_2$ eq. emission saving potential as influenced by cropping system at (a) Hohenschulen and (b) Karkendamm

mono-culture revealed the highest GHG saving potential at both experimental sites, as also reported by Gerin *et al.* (2008).

The comparison of fertiliser types showed less pronounced differences for the emission of greenhouse gases (Figure 2a, 2b). Especially for cropping systems fertilized with biogas residue higher values induced by the direct and indirect emission of N<sub>2</sub>O had been expected. However, no significant differences in direct N<sub>2</sub>O emissions after application of mineral N or biogas residue had been detected (Senbayram *et al.*, 2009). In contrast to the impact of N type, the experimental site (Figure 2b) had a considerable impact. GHG emissions of maize monoculture grown at HS exceeded those at KD by 2000 kg CO<sub>2</sub> eq. ha<sup>-1</sup>. This could be traced back to a soil texture effect, where at the loamy soil site Hohenschulen emissions of N<sub>2</sub>O were at least 3 times higher than at the sandy soil site Karkendamm (Senbayram *et al.*, 2009). It also becomes evident (Figure 2) that the provision of substrates (crop production, direct and indirect N<sub>2</sub>O emission and  $\Delta$  C-humus) accounts for the biggest share of the contents of the GHG emissions.



Figure 2. Components included in the GHG emission calculation for maize monoculture and grassland at Karkendamm (KD) (a) and maize monoculture at Hohenschulen (HS) and KD (b), based on the net energy yields at optimal N input for maize monoculture (151 kg N ha<sup>-1</sup> at HS, and 140 kg N ha<sup>-1</sup> at KD) and grassland at KD (289 kg N ha<sup>-1</sup>)

#### Conclusions

Considering the whole production chain, maize monoculture revealed a higher GHG mitigation potential than the other systems investigated. The type of N-fertilizer had no impact on the GHG emission and mitigation potential, whereas a pronounced influence of local soil conditions was observed.

#### References

Claus S., Wienforth B., Sieling K., Kage H., Taube F. and Herrmann A. (2011) Energy balance of bioenergy cropping systems under the environmental conditions of Schleswig-Holstein. *Grassland Science in Europe* 16, 365–367.

Gericke D. (2009) Measurement and modelling of ammonia emissions after field application of biogas slurries. Doctoral thesis, Christian-Albrechts-Universität zu Kiel, Germany.

Gerin P., Vliegen F. and Jossart J. (2008) Energy and CO<sub>2</sub> balance of maize and grass as energy crops for anaerobic digestion. *Bioresource Technology* 99, 2620–2627.

Senbayram M., Chen R., Mühling K.H. and Dittert K. (2009) Contribution of nitrification and denitrificationderived nitrous oxide emissions from soil after application of biogas waste compared to other fertilizers. *Rapid Communication in Mass Spectrometry* 23, 2489–2498.

# Rooting density of three grass species and eight Lolium perenne cultivars

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## Abstract

Grassland with deeper and denser rooting can take up more nutrients and water, which increases productivity and reduces nutrient losses. The choice of specific grass species and/ or cultivars could be an effective management tool to enhance rooting depth and density. This hypothesis was tested in two field experiments on sandy soil. In experiment I, the root mass, root length and root diameter of *Lolium perenne* (Lp), *Festuca arundinacea* (Fa) and *Dactylis glomerata* (Dg) were compared. In experiment II, the same was measured in eight selected cultivars of Lp, contrasting in terms of ploidy (diploid, tetraploid), production level (high, low) and earliness of production (early, late). In experiment I the results showed that the root biomass in the deeper soil layers (16–24 cm and 24–32 cm) of Fa was significantly higher compared to Lp or Dg. In experiment II, the root length density of the eight cultivars varied between 14.5 to 27.9 cm cm<sup>-3</sup>. Diploid cultivars had a higher root mass, higher root length and lower mean root diameter than tetraploid cultivars. It can be concluded that using *Festuca arundinacea* and/or diploid *Lolium perenne* cultivars in grass mixtures can increase rooting depth and density.

Keywords: root mass, root length, grass species, Lolium perenne cultivars

## Introduction

Increasing the nutrient uptake of grassland can reduce environmental pollution. Although grasses already have a dense rooting system, there are indications that increased rooting can still increase uptake of nutrients and production (Van Eekeren *et al.*, 2011; Van Loo *et al.*, 2003). Therefore, it is important to investigate which management practices promote deeper and denser rooting of grassland. One of those is the use of genetic variation among different grass species or cultivars (Bonos *et al.*, 2004; Crush *et al.*, 2007). In this paper, we describe the results of two experiments in which the distribution of root mass and density of three grass species and eight *Lolium perenne* cultivars were investigated.

## Materials and methods

In experiment I, *Lolium perenne* (Lp) cv. Bargala, *Festuca arundinacea* (Fa) cv. Barolex and *Dactylis glomerata* (Dg) cv. Ambassador were sown in april 2007 on a sandy soil. In October 2010, root samples were taken in four soil layers: 0-8, 8-16, 16-24 and 24-32 cm. Per plot and per layer, three soil cores (8.5 cm diameter, 8 cm depth) were taken and pooled to one sample. The fresh samples were washed through a sieve (mesh size 2 mm) and non-root particles were removed. Root length per diameter class was measured by image analysis using WinRHIZO (Bouma *et al.*, 2000) (scan resolution 400 dpi; filter of particles smaller than 0.2 cm<sup>2</sup> and with a length / width ratio lower than 6) for calculating root length density (RLD). The root dry matter (DM) was determined after drying, first at 70 and then at  $105^{\circ}$ C.

In experiment II, eight *Lolium perenne* cultivars were selected (Table 1) from an experiment with more than 50 Lp cultivars sown in 2005 on a sandy soil. Sampling method and analysis procedure were the same as for experiment I, with the difference that only three soil layers were sampled: 0–8, 8–16 and 16–24 cm.

Culti		Cultivar characteristic						
Var nr	Dlaidy	Production category	Earliness category					
vai III.	Plotdy	(yield index)	(yield index 1 <sup>st</sup> cut)					
1	Diploid	High (102%)	Early (111%)					
2	Diploid	High (100%)	Late (84%)					
3	Diploid	Low (97%)	Early (96%)					
4	Diploid	Low (93%)	Late (89%)					
5	Tetraploid	High (103%)	Early (123%)					
6	Tetraploid	High (101%)	Late (91%)					
7	Tetraploid	Low (99%)	Early (104%)					
8	Tetraploid	Low (99%)	Late (82%)					

Table 1. Characteristics Lolium perenne cultivars in experiment II

#### **Results and discussion**

In experiment I, the total root DM and mean RLD in 0–32 cm were not significantly different between grass species. Significant differences appeared in the soil layers below the upper 8 cm (Figure 1). Overall, Fa developed more roots in the lower soil layers than Lp and Dg. This was confirmed by the significant lower percentage of total root DM in the upper 8 cm (P = 0.010) for Fa (65%) compared to Lp (83%) or Dg (76%). Analysis of the mean root diameter over the whole soil layer indicated a highly significant species effect (P < 0.001), with Dg having the thinnest roots (0.25 mm) and Fa the thickest (0.32 mm). In experiment II, significant differences in rooting parameters were found between cultivars (Figure 2).



Figure 1. Root mass (kg DM ha<sup>-1</sup>) and root length density (cm cm<sup>-3</sup>) of the grass species *Lolium perenne* (Lp), *Festuca arundinacea* (Fa) and *Dactylis glomerata* (Dg) over four soil-depth layers (Experiment I). Different letters indicate a significant difference between means of grass species in a given soil layer (P < 0.005)



Figure 2. Cumulated root mass (kg DM ha<sup>-1</sup>) and mean root length density (cm cm<sup>-3</sup>) of eight *Lolium perenne* cultivars over three soil-depth layers (Experiment II). Error bars represent standard errors in the soil layer 0–24 cm

Rooting parameters were influenced by ploidy: in soil layer 0-24 cm, diploid cultivars had more root DM, higher RLD and lower mean root diameter (P = 0.005) than tetraploid cultivars (Table 2).

		· · · ·	1 0 5			
		Root mass	P-value	Root length density	<i>P</i> -value	
		(kg DM ha <sup>-1</sup> )	i vuide	$(cm \ cm^{-3})$	i vulue	
Ploidy	Diploid	3624	0.004	24.4	< 0.001	
	Tetraploid	2949		18.5		
Production	High	3243	0.686	20.8	0.432	
	Low	3331		22.1		
Earliness	Early	3137	0.168	20.4	0.211	
	Late	3436		22.4		

Table 2. Means of root DM and RLD (0-24 cm) per category (Experiment II)

## Conclusions

Based on the results of these two experiments, it can be concluded that the root mass and density in grassland can be influenced by the choice of grass species and cultivars. *Festuca arundinacea* had more roots in deeper soil layers than *Lolium perenne* and *Dactylis glomerata*. Diploid cultivars of *Lolium perenne* had a higher root mass and a higher root length density than tetraploid cultivars.

## References

Bouma T.J., Nielsen K.L. and Koutstaal B. (2000) Sample preparation and scanning protocol for computerised analysis of root length and diameter. *Plant and Soil* 218, 185–196.

Bonos S.A., Rush D., Hignight K. and Meyer W.A. (2004) Selection for deep root production in tall fescue and perennial Ryegrass. *Crop Science* 44, 1770–1775.

Crush J.R., Easton H.S., Waller J.E., Hume D.E. and Faville M.J (2007) Genotypic variation in patterns of root distribution, nitrate interception and response to moisture stress of a perennial ryegrass (*Lolium perenne* L.) mapping population. *Grass and Forage Science* 62, 265–273.

Van Eekeren N., Deru J., De Boer H. and Philipsen B. (2011) *Back to the grass roots: Improving the nutrient use efficiency with a deeper and denser root system* (in Dutch). Louis Bolk Institute, the Netherlands, 32 pp. Van Loo E.N., Dolstra O., Humphreys M.O., Wolters L., Luessink W., de Riek J. and Bark N. (2003) Lower nitrogen losses through marker assisted selection for nitrogen use efficiency and feeding value in ryegrass (Nimgrass). *Vorträge für Pflanzenzüchtung* 59, 270–279.

# Grassland renovation as a possibility for increasing nitrogen efficiency

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# Abstract

The use of nitrogen in permanent grassland is usually characterized by a low efficiency and depends on the botanical composition of the grassland swards. A high percentage of herbs decreases this efficiency. Grassland renovation, where applicable, can increase the percentage of grasses as one possibility to improve the situation. The methods of grassland improvement, however, differ widely in their effects on N utilisation and economic efficiency. Therefore, common methods of grassland renovation – total renovation after chemical treatments with glyphosate, total renovation after mechanical sward destruction, frequent over-seeding and reseeding via slot drilling technique were compared in a field experiment. DM and nitrogen yields are measured and the costs for the different grassland renovation methods were determined. After 3 experimental years, total renovation, at least if implemented in spring-time, seems not to be the best method for grassland improvement.

Keywords: grassland improvement, slot seeder, methods, permanent grassland, nitrogen

# Introduction

Permanent grassland is a resource that is still not optimally used for protein production and therefore grassland improvement perfectly meets the new political targets in Germany for farm protein supply without additional purchased soya. Possibilities for improving protein yields from grassland are, in general, early cutting, the increase of nitrogen fertilisation, an increase of the nitrogen-efficiency and an improvement of the botanical composition via higher percentages of legumes. Unfortunately, the swards of permanent grassland in South Germany especially often include high percentages of herbs and weeds with only slight reactions to fertilizer nitrogen (Elsaesser, 2005), whereas grasses respond more to N fertilisation. Grassland renovation in order to improve the proportion of grasses can therefore be a possibility to increase the grassland productivity. However, in spite of the expectation of increased DM yields in the years following the treatment (Taube and Conijn, 2007), reseeding of permanent swards might not always result in net benefits (Soegaard et al., 2007). Furthermore, total costs of renovation with regard to the lack of yield in the first year after treatment have - so far - not been calculated exactly. For the evaluation of the success of grassland renovation methods, the improvement of the botanical composition has to be taken into account. Therefore, suitable methods for the agricultural practice are to be investigated.

## Materials and methods

The experiment was established in 2009 in a randomized complete block design with 3 replications on a permanent grassland in Aulendorf (600 m a.s.l.; 950 mm annual rainfall; plot size 100 m<sup>2</sup>). The treatments were as follows: T1. untreated control; T2. total renovation at second growth after spraying with 4 kg ha<sup>-1</sup> Roundup Ultra max (45% glyphosate) and direct drilling 3 weeks later; T3. total renovation at second growth after spraying a mixture of 1 kg ha<sup>-1</sup> Roundup Ultra max(45% glyphosate and 10 kg ha<sup>-1</sup> ammonium sulphate (SSA) ammonium sulphate and direct drilling 3 weeks later; T4. reseeding with a Vredo

slot seeder and 25 kg ha<sup>-1</sup> seed mixture NSF (*Lolium perenne* 48%, *Phleum pratense* 24%, *Poa pratensis* 16% and *Trifolium repens* 12%) at the second growth; T5. two times overseeding per year with disc broadcaster of 5 kg ha<sup>-1</sup> seed mixture NSF at second and fourth growth; T6. total renovation after tillage with rototiller. Each renovation treatment (T2, T3 and T6) was sown with 35 kg ha<sup>-1</sup> of seed mixture GSWI (*Lolium perenne* 59%, *Poa pratensis* 13%, *Phleum pratense* 19%, *Trifolium repens* 9%). All plots were regularly mown 5 times per year and were fertilized with 120, 260 and 240 kg ha<sup>-1</sup> nitrogen as calcium ammonium nitrate per year in 2009, 2010 and 2011, respectively. In the first year, the totally renewed parcels could only be mown 3 times. DM yields, crude protein contents (CP) and net energy yields were investigated and the change of botanical composition was determined (method "Ertragsanteilschätzung" after Klapp, 1949). N efficiency was determined as kg DM kg N<sup>-1</sup> fertilized.

#### **Results and discussion**

After three experimental years the total renovation of permanent grassland swards (T2, T3 and T6) did not produce the highest DM and crude protein (CP) yields. Moreover, the losses in yield after total destruction of the swards during the first year were too high and could not be compensated (Figure 1). Highest DM yields (analysed for 3 years) were observed after reseeding (T4 and T5), whilst over-seeding (T5) resulted in still higher yields than reseeding using the slot-seeder technique with `Vredo` (T4). Totally renewed swards (T2, T3 and T6) yielded on average 7 t DM ha<sup>-1</sup> less than reseeded swards.



Figure 1. Comparison of mean annual and total DM yields (sum of 2009–2011) in t DM ha<sup>-1</sup> (different letters give statistically different results with P < 0.05)

The newly sown swards were neither able to compensate for the DM losses after the treatments (Figure 1), nor to have better swards with higher percentages of grasses or legumes (Table 2). The negative results for totally renewed grassland could also be observed for the crude protein, which has been analysed for 2 experimental years (Table 1). Reseeded grassland, however, had on average 0.3-0.5 t ha<sup>-1</sup> higher CP yields. Nitrogen efficiency expressed as kg DM produced per kg nitrogen fertilizer was highest for frequent over-seeding (T5) due to a gentle treated grassland sward. The regrowths after total renovation had lower protein yields and lower nitrogen efficiency in the first year, which is equalized in the next years (Table 1).

Focusing on the botanical composition of the grassland swards, it was obviously not possible to establish swards with higher proportions of white clover via grassland renovation compared with the reseeded swards. On the contrary, the highest percentages of white clover could be obtained with T5: over-seeding (Table 2).

Table 1. Crude protein yields (2009–2010) and N efficiency (means; different letters symbolize statistical differences: P < 0.05)

Treatment	CF	$P(t ha^{-1})$	N <sub>eff</sub> (kgDM kgN <sup>-1</sup> )		
meatment	2009	2010 mean	2009	2010	2011
T1 untreated control	1.66 b	2.27 a 1.97 a	8.6 a	5.0	5.3
T2 total renovation with 4 kg ha <sup>-1</sup> Roundup Ultra max	0.92 c	2.40 a 1.66 a	4.2 b	5.2	5.3
T3 total renovation with 1 kg $ha^{-1}$ Roundup + 10 kg $ha^{-1}$ ammonium sulphate	0.99 c	2.36 a 1.67 a	4.2 b	5.0	5.1
T4 1 x reseeding with Vredo slot seeder	1.71 ab	2.48 a 2.09 a	8.2 a	5.5	5.6
T5 frequent overseeding (2 x per year)	2.03 a	2.43 a 2.23 a	9.8 a	5.2	5.6
T6 total renovation with rototiller	1.08 c	2.21 a 1.65 a	4.8 b	5.1	5.3
LSD	0.356	0.277 0.697	1.7	ns	ns

Table 2. Percentages of legumes and grasses in the grassland swards after 3 experimental years (September 2011) (different letters symbolize statistical differences P < 0.05)

Treatments	T1	T2	T3	T4	T5	T6
White clover	5.0 abc	5.3 ac	5.3 ac	5.3 abc	9.7 c	2.3 ab
Grasses	58.3 b	44.3 a	70.7 c	55.7 ab	58.3 abc	55.3 ab

#### Conclusions

Methods of grassland renovation are used both in order to improve botanical composition of grassland as well as to increase the DM and protein yields. However, these effects are not guaranteed to meet expectations. In our experiment on permanent grassland, reseeding seems to be more successful than total renovation if the date of total destruction of grassland is in spring. Yield losses from the first and second growth in the year of establishment could not be compensated. Reseeding, as well as the slot-drilling method or frequent over-seeding, seem to be the best and most economic treatments to improve the swards of permanent grasslands. This could be found for both DM and crude protein production. Total renovation was able to increase the percentage of grasses.

#### References

Elsäßer M. (2005) Performance of a reseeded grassland (Trisetetum) in Germany. *Grassland Science in Europe* 10, 477–480.

Klapp E. (1949) Landwirtschaftliche Anwendung der Pflanzensoziologie. *Verlag Eugen Ulmer*, Stuttgart. Soegaard K., Gierus M., Hopkins. A. and Bommelé L. (2007) Effects of grassland renovation on crop and animal performance. In: *Grassland resowing and grass-arable crop rotations*. Third and fourth workshop of the EGF working group. Report 148. Wageningen Plant Research International, 95–105.

Taube F. and Conijn J. (2007) Grassland renovation in Northwest Europe: current practices and main agronomic and environmental questions. In: *Grassland resowing and grass-arable crop rotations*. Third and fourth workshop of the EGF working group. Report 148. Wageningen Plant Research International, 35–38.

# Root biomass and carbon storage in differently managed multispecies temporary grasslands

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## Abstract

Species-rich grasslands may potentially increase carbon (C) storage in soil, and an experiment was established to investigate C storage in highly productive temporary multi-species grasslands. Plots were established with three mixtures: 1) a herb mixture containing salad burnet (*Sanguisorba minor* L.), fenugreek (*Trogonella foenum-gruecum*), chicory (*Chicorium intybus* L.), caraway (*Carum carvi* L.), birdsfoot trefoil (*Lotus corniculatus* L.), chervil (*Anthriscus cerefolium* L.), plantain (*Plantago lanceolata* L.), lucerne (*Medicago sativa* L.) and melilot (*Melilotus officinalis*), 2) 50% of the herb mixture, and 30 5% of the herb mixture and 95% of the white clover-ryegrass mixture. Management factors were number of cuts per year and fertilizer application. Aboveground biomass increased considerably with increasing content of herbs and with fertilizer application in plots with a 4-cut strategy. With a 6-cut strategy without fertilizer, herbs had no effect on the aboveground biomass. In the herb mixture, biomass of small roots was lower than in mixtures with white clover and ryegrass. There was a tendency towards increased CO<sub>2</sub> evolution following cultivation of multispecies grasslands.

Keywords: multispecies mixtures, herbs, white clover, ryegrass, root, CO<sub>2</sub> emission

## Introduction

In grasslands most C originates from roots and total allocated C increases with plant species richness (Adair *et al.*, 2009). The larger roots seem to be more important than small roots in enhancement of the C pools (Rasmussen *et al.*, 2010). However, the storage of C in soils depends on both the inputs and the decomposition rate, which is especially important in farming systems with frequent grassland cultivation. We investigated above and belowground biomass in differently managed multispecies mixtures and  $CO_2$  emission upon grassland cultivation.

## Materials and methods

A plot experiment was established with three mixtures: 1) a herb mixture containing salad burnet, fenugreek, chicory, caraway, birdsfoot trefoil, chervil, plantain, lucerne and melilot; 2) 50% of the herb mixture and 50% of a white clover-perennial ryegrass mixture; and 3) 5% of the herb mixture and 95% of the white clover-ryegrass mixture. Also, some herbs were established in pure stands. All mixtures were managed with 4 or 6 cuts per year, with and without fertilizer application (200 kg N ha<sup>-1</sup>via cattle slurry) for the 4-cut system. The pure stand was managed with 4 cuts and without fertilizer. In the spring of the third production year, aboveground biomass was determined by harvesting plots of  $1.5 \times 12$  m, and belowground biomass was determined by wet sieving of eight soil samples per plot from three depths (0–10, 10–20 and 20–50 cm) sampled with an 8.75 cm inner-diameter auger. Furthermore, CO<sub>2</sub> release following simulated cultivation was investigated in an incubation experiment for soils from 0–10 and 10–20 cm depths.

#### **Results and discussion**

Aboveground biomass increased considerably with increasing content of herbs in the mixture and also with fertilizer application in plots with a 4-cut strategy (Table 1). With a 6-cut strategy, aboveground biomass was much depressed compared to the 4-cut strategy; in the previous years this depression was only noticed in the 100% herb mixture (Mortensen *et al.*, 2012). The herb mixture was dominated by lucerne, most pronounced without fertilizer, and caraway, most pronounced with fertilizer application.

Table 1. Aboveground biomass and botanical composition of swards with different herb seeding rates, manure application and cutting frequency in spring cut. Biomass values with different letters are statistically different (P < 0.05). Annual biomass production in Mortensen *et al.* (2012)

Herbs in mix	Manure applied	Cuts per yea	Bio- mass r t DM	Rye- grass	White Clover	Lu- cerne	Cara- way	Chi- cory	Salad burnet	Plan- tain	Birds- foot trefoil	Un- sown
			ha <sup>-1</sup>			Pro	portion	of dry	weight (	$\%)^{1}$		
5%	0 N	4	2.6 <sup>d</sup>	61	32	4	3	0	0	0	0	0
50%	0 N	4	3.6°	34	10	40	14	1	0	0	0	0
100%	0 N	4	4.7 <sup>b</sup>	0	0	77	17	1	2	0	1	2
5%	200 N	4	3.2 <sup>cd</sup>	74	22	0	5	0	0	0	0	0
50%	200 N	4	4.4 <sup>b</sup>	37	7	26	30	0	0	0	0	1
100%	200 N	4	5.9ª	0	0	30	67	1	1	0	0	1
5%	0 N	6	1.8 <sup>e</sup>	59	39	0	0	0	0	0	0	2
50%	0 N	6	1.7 <sup>e</sup>	61	33	0	3	0	0	1	0	2
100%	0 N	6	1.6 <sup>e</sup>	0	0	15	39	5	8	6	8	20

<sup>1</sup>Chervil, melilot and fenugreek were not present at all.

Total root biomass (small and large roots at all depths) was not significantly affected by treatments or by species type in the pure stand experiment (Figure 1). However, in specific size classes and depths differences appeared. Thus, in the 100% herb mixture the biomass of small roots (<8 mm) in the top layer were significantly lower (P < 0.001) than in mixtures with white clover and ryegrass, and similarly the root biomass in this fraction was lower without fertilizer application (P < 0.01). The biomass of large roots (>8 mm) in mixtures with herbs showed considerable variation probably as a result of more taproots and there was a non-significant tendency towards increased biomass in the large root fraction with increasing herb content. This was probably related to the high contents of lucerne and caraway both having significantly larger root biomass in 10–20 and 20–50 cm in the pure stand plots of these species.



Figure 1. Root biomass at different depth of swards with different herb seeding rate (5, 50 or 100%), manure application (0 or 200N) and cutting frequency (4or 6-cut) and of selected species in pure stand in a separate experiment. Error bars: SE



Figure 2. Accumulated  $CO_2$  evolved from soil during incubation at 20°C for 97 days. Error bars: SE. Unreplicated results for soil from plots with species in pure stand

Release of CO<sub>2</sub> during incubation following simulated cultivation showed significantly (P < 0.001) higher mineralization in the topsoil compared to 10–20 cm (Figure 2), indicating larger C deposition in this layer of the grassland soil where a huge part of root biomass was also present. There was no effect of fertilization and cutting management on CO<sub>2</sub> release following cultivation, but in the 10–20 cm layer CO<sub>2</sub> evolution from the soil of the 100% herb mixture was significantly increased compared to the mixtures with grass-clover (P < 0.05). This indicates that at least in the plough layer (0–20 cm) the extra C sequestered by temporary multispecies grasslands may to some extent be accompanied by increased mineralization following cultivation.

#### Conclusions

In the present experiment, inclusion of herb species in grasslands was shown to increase yield stability (Mortensen *et al.*, 2012). Furthermore, the results presented here have indicated some potential of species known to have deeper and denser rooting systems to increase belowground biomass, an important asset for C sequestration in grasslands. However, in mixed cropping systems with temporary grassland it is important to know if differences in root composition affect the rate of decomposition of soil organic matter upon cultivation to evaluate the net C storage of the cropping system. This experiment gave an indication of increased CO<sub>2</sub> evolution following cultivation of multispecies grasslands, but this needs further investigation over longer time periods and with focus also on soil below the plough layer.

#### References

Adair E.C., Reich P.B., Hobbie S.E. and Knops J.M.H. (2009) Interactive effects of time, CO<sub>2</sub>, N, and diversity on total belowground carbon allocation and ecosystem carbon storage in a grassland community. *Ecosystems* 12, 1037–1052.

Mortensen T., Søegaard K. and Eriksen J. (2012) Effect of seed mixture composition and management on competitiveness of herbs in temporary grasslands. *Grassland Science in Europe* (this issue).

Rasmussen J., Eriksen J., Jensen E.S. and Jensen H.H. (2010) Root size fractions of ryegrass and clover contribute differently to C and N inclusion in SOM. *Biology and Fertility of Soils* 46, 293–297.

# Soil and pasture evolution of zinc in a silvopastoral system developed under *Quercus rubra* L. after fertilization with different doses of sewage sludge

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# Abstract

One option adopted in many countries around the world is the use of sewage sludge as fertilizer in agriculture. However, the use of this residue as fertilizer must take into consideration its heavy metal concentration (mainly Zn), which is higher than that normally found in soils and may lead to crop toxicity and environmental degradation. The aim of this study was to evaluate the effects of different doses of municipal sewage sludge (100, 200 and 400 kg total N ha<sup>-1</sup>) on the total and available Zn concentrations in soil and the Zn levels of pasture compared to control treatment (no fertilization) in a silvopastoral system under *Quercus rubra* L., and a sown sward with *Dactylis glomerata* L., *Lolium perenne* L. and *Trifolium repens* L. during the four years after the study was established in Galicia (Spain). The results showed that fertilization with sewage sludge increased the concentration of Zn in the soil and plants, mainly when high doses of sewage sludge were applied (400 kg total N ha<sup>-1</sup>), but never exceeded the maximum values set by Spanish regulations and did not cause harmful effects on plants and animals.

Keywords: agroforestry, heavy metals, waste, sowing, afforestation

## Introduction

Many studies have shown that the use of sewage sludge as fertilizer in silvopastoral systems increases tree and pasture production (Ferreiro-Domínguez *et al.*, 2011; Mosquera-Losada *et al.*, 2011). However, the application of sewage sludge to soil can pose a threat to the environment, with the major concern arising from the fact that sewage sludge contains a relatively higher concentration of heavy metals (mainly Zn) than that normally found in soils (Smith, 1996), regulated in Spain by the R.D. 1310/1990 and by the European Directive 86/278/EEC. Moreover, when sewage sludge is used as fertilizer it is important to apply an adequate dose of this residue. A sewage sludge application rate exceeding the crop needs could result in nitrate contamination of the ground water by leaching (EPA, 1994). The objective of the present study was to evaluate the effects of different doses of sewage sludge (100, 200 and 400 kg total N ha<sup>-1</sup>) compared with a control treatment (no fertilization) on the total and available Zn concentration in soil, and on the Zn levels of pasture in a silvopastoral system under *Quercus rubra* L.

# Materials and methods

The experiment was conducted on an area of agriculturally abandoned land in Lugo (Galicia, NW Spain) at an altitude of 550 m a.s.l. The pasture was sown with a mixture of *Dactylis glomerata* L. cv. Artabro (12.5 kg ha<sup>-1</sup>), *Lolium perenne* L. cv. Brigantia (12.5 kg ha<sup>-1</sup>) and *Trifolium repens* L. cv. Huia (4 kg ha<sup>-1</sup>) in autumn 2001, with bare-rooted plants of *Quercus rubra* L. being planted at a density of 1,112 trees ha<sup>-1</sup>. The experimental design was a randomized complete block with three replicates and four treatments. Each experimental unit had an area of 144 m<sup>2</sup> and 25 trees planted with an arrangement of 5×5 stems, forming a perfect

square. Treatments consisted of no fertilization (0N) and fertilization with anaerobically digested sludge with an input of 100 kg total N ha<sup>-1</sup> (100N) 200 kg total N ha<sup>-1</sup> (200N) and 400 kg total N ha<sup>-1</sup> (400N) in March 2002 and 2003. The calculation of the required amounts of sludge was conducted according to the percentage of total nitrogen (EPA, 1994) and taking into account the Spanish regulation (R.D.1310/1990) regarding the heavy metal concentration for sewage sludge application. To estimate the total and available Zn concentration in soil and the pasture Zn content, in March 2003 and in January 2004, 2005 and 2006, a composite soil sample per plot was collected at a depth of 25 cm, as described in the RD 1310/1990. Four samples of pasture were randomly taken per plot ( $0.3 \times 0.3 \text{ m}^2$ ) in June 2002, in July and December 2003, in June, July and December 2004 and in May, July and December 2005 (only significant results are shown). Soil total (CEM, 1994), were estimated in the laboratory. Soil variables were analysed with ANOVA (proc glm procedure). Means were separated by using LSD test, if ANOVA was significant (SAS, 2001).

#### **Results and discussion**

The soil total (2003: 12.78<sup>b</sup>; 2004: 14.06<sup>b</sup>; 2005: 11.93<sup>b</sup>; 2006: 26.77<sup>a</sup> expressed as mg kg<sup>-1</sup>) (P < 0.01) and available Zn (2003: 1.07<sup>b</sup>; 2004: 5.21<sup>a</sup>; 2005: 3.83<sup>ab</sup>; 2006: 5.92<sup>a</sup> expressed as mg kg<sup>-1</sup>) (P < 0.05) (in all cases different superscript letters indicate significant differences between years) were significantly higher in 2006 than in 2003. This result could be explained by an increase in N mineralisation caused by the higher soil pH in 2006 than in 2003, and by an increment of precipitation and temperatures at the end of the studied years compared with the mean for the last 30 years (Ferreiro-Domínguez *et al.*, 2011). N mineralization is indicative of the sewage sludge incorporation into soil and may have promoted heavy metal release from the sludge to the soil. On the other hand, it should be noted that all values of total Zn in the soil of this experiment (11.93–26.77 mg kg<sup>-1</sup>) were very low compared to the Spanish regulation limits (150 mg kg<sup>-1</sup>) (R.D.1310/1990). This may be explained by the fact that this study was located in an area without nearby pollution sources and that the soil had initial low levels of this element (11.9 mg Zn kg<sup>-1</sup>).

Regarding to the effect of the treatments, in Figure 1 shows a significantly increased total



Figure 1. Mean soil total and available zinc in 2003, 2004, 2005 and 2006. 0N: 0 kg total N ha<sup>-1</sup>; 100N: 100 kg total N ha<sup>-1</sup>; 200N: 200 kg total N ha<sup>-1</sup> and 400N: 400 kg total N ha<sup>-1</sup>. Different letters indicate significant differences between treatments. Vertical lines indicate mean standard error

Zn concentration in soil (P < 0.05) when a high (400N) dose of sewage sludge was applied compared with low (100N) dose and with the no fertilization (0N) treatment; this trend was also observed on the concentration of available Zn (P > 0.05). This result could be due to the Zn being present in the sewage sludge in greater amounts than the background values

in the receiving soil (Smith, 1996) and because the amount of Zn applied to the soil during 2002 and 2003 with the high (16.85 kg Zn  $ha^{-1}$ ) dose of sewage sludge was higher than with the low (4.21 kg Zn  $ha^{-1}$ ) and medium (8.05 kg Zn  $ha^{-1}$ ) doses.

Finally, an increment of soil total Zn and available Zn increased the Zn concentration in the pasture (Krebs *et al.*, 1998) as observed in Table 1, in which it is apparent that the high (400N) dose of sewage sludge implied higher concentrations of Zn in pasture than the no fertilization (0N) but not exceeding the levels of 100 and 400 mg Zn kg<sup>-1</sup>, which are considered excessive and toxic, respectively, for plants (Smith, 1996). With regard to animals, the maximum Zn in the forage concentrations established by NRC (1980) for bovines (500 mg Zn kg<sup>-1</sup>) and ovines (300 mg Zn kg<sup>-1</sup>) were never exceeded, which indicated that the pasture of this experiment was suitable for animal consumption.

Table 1. Concentrations of Zn in pasture (mg kg<sup>-1</sup>). 0N: 0 kg total N ha<sup>-1</sup>; 100N: 100 kg total N ha<sup>-1</sup>; 200N: 200 kg total N ha<sup>-1</sup> and 400N: 400 kg total N ha<sup>-1</sup>. Different letters indicate significant differences between treatments in each harvest. \*\*\* P < 0.001; \*\* P < 0.01; \* P < 0.05; SEM: mean standard error

		Plant zinc	Voor offoot	SEM		
	0N	100N	200N	400N	Teal effect	SEM
Jul-03	4.75 b	11.4 b	17.4 ab	29.4 a	*	3.19
Jun-04	12.93 b	16.83 ab	14.83 b	20.77a	*	1.39
May-05	23.63 b	34.84 a	38.83 a	34.74 a	*	2.17
Jul-05	20.87 c	27.35 b	28.88 b	36.52 a	**	1.81
Feb-06	41.18 c	56.47 b	67.36 ab	75.99 a	***	3.17

## Conclusions

The fertilization with sewage sludge increased the concentration of Zn in the soil and plants, mainly when high doses of sewage sludge were applied (400 kg total N ha<sup>-1</sup>), but never exceeded the maximums set by Spanish regulations and did not cause harmful effects on plants and animals. Therefore, the use of high quality sewage sludge as fertilizer may improve the productivity of the herbaceous and tree components of silvopastoral systems without creating environmental hazards.

#### References

CEM (1994) Microwave digestion applications manual, CED (ed.), USA, 112 pp.

EPA (Environmental Protection Agency) (1994) Land application of sewage sludge. A guide for land appliers on the requirements of the federal standards for the use of disposal of sewage sludge 40 CFR Part 503, Environment Protection Agency, Washington D.C., USA.

Ferreiro-Domínguez N., Rigueiro-Rodríguez A. and Mosquera-Losada M.R. (2011) Response to sewage sludge fertilisation in a *Quercus rubra* L. silvopastoral system: Soil, plant biodiversity and tree and pasture production. *Agriculture, Ecosystems and Environment* 144, 49–57.

Krebs R., Gupta S.K., Furrer G. and Schulin R. (1998) Solubility and plant uptake of metals with and without liming of sludge-amended soil. *Journal of Environmental Quality* 27, 19–23.

Mehlich A. (1985) Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis* 15, 1409–1416.

Mosquera-Losada M.R., Morán-Zuloaga D. and Rigueiro-Rodríguez A. (2011) Effects of lime and sewage sludge on soil, pasture production, and tree growth in a six-year-old *Populus canadensis* Moench silvopastoral system. *Journal of Plant Nutrition and Soil Science* 174, 145–153.

NRC (National Research Council) (1980) *Mineral Tolerance of Domestic Animals*, National Academic Press, Washington, USA, 155 pp.

SAS (2001) SAS/Stat User's Guide: Statistics, SAS Institute Inc., Cary, NC, USA, 1223 pp.

Smith S.R. 1996. Agricultural Recycling of Sewage Sludge and the Environment, CAB International, Wallingford, UK.

# Soil organic carbon degradation: is silage maize unfairly overestimated?

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# Abstract

Land use change represents a major source of anthropogenic induced greenhouse gas emissions. A monitoring study was conducted to quantify the impact of land use systems on soil organic carbon stocks on various sites throughout Schleswig-Holstein, Northern Germany. Results revealed higher SOC stocks under grassland compared to arable cropping. Longterm maize monoculture, however, did not show lower C sequestration than arable rotations with or without maize.

Keywords: silage maize, monoculture, rotation, permanent grassland, soil carbon

# Introduction

Soil organic carbon (SOC) is a main determinant of soil quality and ecosystem functioning. Silage maize cultivation is commonly regarded to cause a stronger decrease in SOC stocks compared to various other crops, especially if grown in monoculture (VDLUFA, 2004). Vertès and Mary (2007), for instance, found a close relationship between the grassto-maize ratio and soil organic matter (SOM) loss in a 27-yr field experiment including various grass/maize rotations. The availability of long-term field experiments to support this hypothesis, however, is limited. The objective of the present study therefore was to analyse the relationship between land use and C sequestration for different landscapes in Schleswig-Holstein, Northern Germany.

## Materials and methods

Based on a monitoring study, SOC stocks of different land use systems (permanent grassland, maize monoculture, maize rotation, arable rotations without maize) were determined on practical farms located in the three main landscapes (Low and High Geest, Eastern Upland, Marsh) of Schleswig-Holstein, Northern Germany. Peat and peaty mineral sites were excluded from the study. Soil samples (0–30 cm, 30–60 cm) were taken in spring/early summer of 2010 on 85 fields belonging to 27 farms. On each farm, permanent grassland (>40 years) served as a reference for the other land use systems. Representative soil samples were dried, sieved (<2 mm), ground to fine powder, and subsequently soil total C was measured using a CN-analyzer (Vario Max CN, Elementar Analysensysteme, Hanau, Germany). The organic C content (Corg) was obtained as the difference of total C and carbonate content (Scheibler, DIN ISO 10693). Soil bulk density was determined at 5, 15, and 45 cm depth for grassland and at 15 and 45 cm depth for arable sites, applying a core method. Statistical analysis comprised pair-wise comparisons of means using t-tests.

# **Results and discussion**

Generally, SOC stocks of cropping systems varied substantially among farms, as indicated by coefficients of variation up to 50%, depending on sample size. As expected, we found a significantly higher SOC stock (t Corg ha<sup>-1</sup>) under permanent grassland than under long-term maize monoculture (>20 years); however, SOC differed only by 20 percent on average (Figure 1).



Figure 1. Organic carbon content (t C ha<sup>-1</sup>) in topsoil and subsoil (0–60 cm) of permanent grassland (>40 years) and long-term maize monoculture (>20 years)

This difference is lower compared to Vertès and Mary (2007), who detected a 30% decrease of SOM after 27 years of maize monoculture, or compared to Guo and Gifford (2002) reporting a 59% SOC stock decline after converting pasture to cropland. Similarly, Hanegraaf *et al.* (2009) found declining SOM for maize monoculture on sandy soils in the Netherlands. In contrast, Kristiansen *et al.* (2005) reported an increase in soil C stocks after 14 years of maize monoculture, probably due to low initial soil C contents. SOC content usually decreases with depth. Correspondingly, we found highest SOC stocks in the top layer (0–30 cm), but still considerable C amounts were detected in the subsoil (30–60 cm), with 40% of the SOC stock in 0–60 cm depth located in the 30–60 cm layer (not presented). It is known from literature that often more than 50% of the SOC stock is found in deep soil horizons (Batjes, 1996), effected by root depth allocation, leaching, abiotic decay conditions, and bioturbation.

If we assume a SOC degradation caused by maize cultivation, an impact of its duration on SOC stocks can be hypothesized. A comparison of short-term (<10 years) with long-term maize monoculture (>20 years) revealed a corresponding trend, but differences were not significant (Figure 2). Differences in SOC stocks between permanent grassland and arable rotations with/without maize were even larger than for long-term maize monoculture, as exemplified in Figure 3 for arable rotations without maize, which on average were characterized by a 30% lower C accumulation compared to permanent grassland.

The causes for this unexpected result are not clear. It might be argued that current maize varieties provide higher above – and below-ground biomass than previously assumed



Figure 2. Organic carbon content (t C ha<sup>-1</sup>) in topsoil and subsoil (0–60 cm) of short-term (<10 years) and long-term maize monoculture (>20 years). Short-term maize fields had been converted from grassland to arable land



Figure 3. Organic carbon content (t C  $ha^{-1}$ ) in topsoil and subsoil (0–60 cm) of permanent grassland (>40 years) and arable rotations without maize (>30 years). E.U: Eastern Upland

(Johnson *et al.*, 2006). Furthermore, in the study area maize fields usually are not ploughed before spring, which avoids excessive SOM mineralization in autumn. Another possible explanation might be that, in preceding decades, maize received higher amounts of farmyard manure than other arable crops. Recent research has highlighted that historic land use and management have a long-lasting imprint on SOC stocks (Schulp and Verburg, 2009). Finally, the overall low C sequestration potential of mineral soils not influenced by groundwater may have contributed. In no case was the SOC content below the threshold set by the cross-compliance commitments. Yet, on groundwater-influenced sites, which had not been included in this study, a larger C sequestration potential and thus higher C loss risk must be assumed.

#### Conclusions

The results of the monitoring study revealed a lower risk of SOC degradation caused by long-term maize cultivation than is commonly assumed, especially on light sandy soils, where a large part of the maize production in Northern Germany is located.

#### References

Batjes N.H. (1996) Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* 47, 151–163.

Hanegraaf M.C., Hoffland E., Kuikman P.J. and Brussaard L. (2009) Trends in soil organic matter contents in Dutch grasslands and maize fields on sandy soils. *European Journal Soil Science* 60, 213–222.

Guo L.B. and Gifford R.M. (2002) Soil carbon stocks and land use change: a meta analysis. *Global Change Biology* 8, 345–360.

Johnson J.M.-F., Allmaras R.R. and Reicosky D.C. (2006) Estimating source carbon from crop residues, roots and rhizodeposits using the national grain-yield database. *Agronomy Journal* 98, 622–636.

Kristiansen S.M., Hansen E.M., Jensen L.S. and Christensen B.T. (2005) Natural <sup>13</sup>C abundance and carbon storage in Danish soils under continuous silage maize. *European Journal of Agronomy* 22, 107–117.

Schulp C.J.E. and Verburg P.H. (2009) Effect of land use history and site factors on spatial variation of soil organic carbon across a physiographic region. *Agriculture, Ecosystems and Environment* 133, 86–97. VDLUFA – Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (2004) Standpunkt Humusbilanzierung. Available at: http://www.vdlufa.de/joomla/Dokumente/Standpunkte/08-humusbilanzierung.pdf. Accessed 10 Nov. 2011.

Vèrtes F. and Mary B. (2007) Modelling the long term SOM dynamics in fodder rotations with a variable part of grassland. In: Chabbi A. (ed.). *Organic Matter Symposium*, 17–19 July 2007, Poitiers, France, 549–550.

# The effects of increasing ditch water levels on grass yield on peat grasslands

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# Abstract

From the 1960s onwards in the Netherlands, many relatively wet peat lands were deep(er) dewatered to a ditch water level of 50 to 60 cm below the surface to improve dairy agriculture. As a consequence of this deeper drainage, peat degradation took place, due to mineralisation, resulting in a relatively rapid land subsidence. An increase of the low ditch level may inhibit subsidence. Although much research has been done in the Netherlands on the effects of deep drainage, hardly any research has been done on the effects of increasing the ditch water levels. In a 6-year trial, the agricultural effects of increasing the ditch levels were examined. This article discusses the effects on gross grass growth at a low or shallow ditch level. In the period 2005–2007, the yield was measured at 3 N levels and a non-fertilized treatment. In the period 2008–2010 the yield was only measured at one level of N fertilization. Although there were differences in yield between years, on average the yield at a low ditch water level was 6% higher than the yield at a shallow ditch level.

Keywords: DM yield, peat soil, increased ditch level, yield reduction

## Introduction

To improve the possibilities for dairy farming in (wet) peat areas in the Netherlands, deeper drainage was considered a suitable solution. In the 1970s ditch levels were lowered to over 60 cm below the surface. The advantages were drier land with less damage caused by trampling or machinery, higher nitrogen (N) output by mineralisation which results in higher yields, better valued grass species (Grootjans *et al.*, 1985) and lower costs (Boxem and Leusink, 1978). But over time it became apparent that there were not only advantages. Due to the mineralisation the field level became lowered by subsidence and dry peat resulted in higher greenhouse gas production (Schrier, 2010). Therefore the policy in parts of the Netherlands has changed in the last five years. The new policy is to increase the ditch levels to prevent any further subsidence.

In this research, a field experiment was set up on the Zegveld research farm, situated in a low peat area. This experiment addressed the question of whether disadvantages due to restoring the previous high ditch and groundwater levels will occur, and how these disadvantages will develop over time. The objectives of the total experiment were to measure yields, trampling and machinery damage, changes in botanical composition, and subsidence of the soil. The experimental period was from 2005 to 2010.

This article describes the effects of changing ditch levels on DM yield. A second article in these proceedings shows the results of the machinery riding damage sub-experiment.

## Materials and methods

Four permanent grassland plots were split up into two equal parts. Two ditch water levels, -50 cm (existing) and -30 cm (new) below the surface, were random assigned to the split parts. This resulted in a design of eight plots (four paired replicates, each with two ditch levels). The experimental layout is given in Figure 1. On every plot, a part was fenced off and used as cutting plot to estimate the DM yield. The remaining part was used for grazing or cutting as



part of the existing dairy grassland management. On the cutting plot, there were sub-plots to estimate the year yield (as the sum of five mown cuts) at three N levels (N1, N2 and N3) in the period 2005–2007 and one N level in the period 2008-2010, and a unfertilized control treatment (0N) The size of the plots was  $10 \times 3$  m. The net strip was 1.50×7 m. The strips were harvested with a Haldrup<sup>TM</sup> mower, developed for experimental fields. Fresh vield was weighed and dried at 70°C to calculate DM content and DM yield, and the samples for 2005-2007 were sent to a laboratory to analyse the N content.

All the plots received a surplus of P and K to prevent a shortage: 70 kg P ha<sup>-1</sup> and 83 kg K ha<sup>-1</sup> was given. The total N supply was 110, 220 and 330 kg N ha<sup>-1</sup> respectively,

Figure 1. Layout of the experiment

for the N1, N2 and N3 treatments in the period 2005–2007. In the period 2008–2010, the N2 treatment received 140 kg N (according to the Dutch N advice for peat soil). Therefore the statistical analysis is also split into two periods.

The differences between the total DM yield (and N yield in the period 2005–2007) as a result of N application and ditch level were statistically analysed using Analysis of variance (ANOVA) with the Genstat program (version 12).

## **Results and discussion**

The weather conditions varied a lot in the period 2005–2010. The years 2005 and 2008 had a surplus of rainfall. In these years, the DM yield was significantly lower. The total DM yield per year as average of four blocks with two replicates each and the statistical results are shown in Table 1. These are the results of the N2 (Dutch N advice) treatments.

0		· <b>T</b>		,			
2005	2006	2007	2008	2009	2010	Av.	Av. N yield
14,903	11,386	13,482	12,616	12,507	10,531	12,417 <sup>a</sup>	414 <sup>a</sup>
13,091	10,994	13,373	11,496	12,123	10,116	$11,745^{b}$	371 <sup>b</sup>
-14%	-4%	-1%	-10%	-3%	-4%	-6%	-11%
13,997	11,190	13,427	12,056	12,315	10,323	12,081	393
	2005 14,903 13,091 -14% 13,997	2005 2006   14,903 11,386   13,091 10,994   -14% -4%   13,997 11,190	2005 2006 2007   14,903 11,386 13,482   13,091 10,994 13,373   -14% -4% -1%   13,997 11,190 13,427	2005 2006 2007 2008   14,903 11,386 13,482 12,616   13,091 10,994 13,373 11,496   -14% -4% -1% -10%   13,997 11,190 13,427 12,056	2005 2006 2007 2008 2009   14,903 11,386 13,482 12,616 12,507   13,091 10,994 13,373 11,496 12,123   -14% -4% -1% -10% -3%   13,997 11,190 13,427 12,056 12,315	2005 2006 2007 2008 2009 2010   14,903 11,386 13,482 12,616 12,507 10,531   13,091 10,994 13,373 11,496 12,123 10,116   -14% -4% -1% -10% -3% -4%   13,997 11,190 13,427 12,056 12,315 10,323	2005 2006 2007 2008 2009 2010 Av.   14,903 11,386 13,482 12,616 12,507 10,531 12,417 <sup>a</sup> 13,091 10,994 13,373 11,496 12,123 10,116 11,745 <sup>b</sup> -14% -4% -1% -10% -3% -4% -6%   13,997 11,190 13,427 12,056 12,315 10,323 12,081

Table 1. Annual yield in kg DM ha<sup>-1</sup> (period 2005–2010) at two ditch levels

In the 'wet' years 2005 and 2008, the DM yield was over 10% lower as result of a higher ditch level. Over the total period the DM yield was 6% lower at a high ditch level.

Looking at the N1–N3 treatments in the period 2005–2007, the DM yield at the N2 object in the high ditch level was 12,500 kg DM  $ha^{-1}$  and was equal to the DM yield on the N1 treatments at the low ditch level. When no nitrogen was used (N0), the difference between N0 at low and high ditch level was about 1,000 kg DM  $ha^{-1}$ , but the difference in N yield was only 30 kg N  $ha^{-1}$ . Schothorst (1982) also found the same differences between an existing high and low ditch level on peat grassland.

It is possible to compensate for the increased DM yield with 110 kg N ha<sup>-1</sup> which is the difference between treatment N1 and N2. This means that the effect of extra N is very low in wet situations. The negative effect of an increase to a higher ditch level will occur directly in the first year. This means that all the negative aspects of a high ditch level, as mentioned in De Vos *et al.* (2006), are also valid for a restoration of the ditch level in the first year after the increase. Considering the lower yield at a high ditch level, it seems that this result is due to there being less mineralisation on the treatments at a high ditch level. Looking at the N yield, the difference at the N0 was 30 kg and at N2 40 kg N ha<sup>-1</sup>. So it is not only mineralisation is also less. These effects will also occur directly after setting up the ditch level, and they were also found in earlier research on low ditch levels (De Vos *et. al.*, 2006). Besides a lower DM yield, the land will be wetter at high water levels, which means that the bearing capacity will be less, especially in wet periods. This also means also there will be fewer possibilities for grazing because of the risk of trampling damage and a greater chance of damage from machinery.

#### Conclusions

Increasing a low ditch level to a high ditch level reduces the yearly DM yield on average by 6%. The N yield (apparent N supply; ANS) of an unfertilized treatment was 30 kg ha<sup>-1</sup> lower after increasing the ditch level. The difference in yield can be compensated by applying 110 kg ha<sup>-1</sup> extra N.

#### References

Boxem Tj. and en Leusink A.W.F. (1978) Ontwatering van veengrasland. PR-publikatie ll.Lelystad (summary in English).

Grootjans A.P., Schipper P.C. and Van der Windt H.J. (1985) Influence of drainage on N-mineralization and vegetation response in wet meadows: I *Calthion palustris* stands. *Acta Oecologia* 6, 403–417.

Schothorst C.J. (1982) Drainage and behaviour of peat soils. In: Bakker H. de and van den Berg M.W. (eds). *Proceedings of the symposium on peat lands below sea level*. International Institute for Land Reclamation and Improvement/ILRI, P.O. Box 45, 6700 AA Wageningen.

Schrier-Uijl A.P. (2010) Flushing meadows. The influence of management alternatives on the greenhouse gas balance of fen meadow areas. *Thesis* Wageningen University.

Van Beek C.L., Hummelink E.W.J., Velthof G.L. and Oenema O. (2004) Denitrification rates in relation to groundwater level in a peat soil under grassland. *Biology and Fertility of Soils* 39(5), 329–336.

Vos J.A. de, Van Bakel P.J.T., Hoving I.E. and Conijn J.G. (2006) Waterpas-model: A predictive tool for water management, agriculture, and environment. *Agricultural Water Management* 86 (1–2), 187–195.

# Genetic approaches to increase phosphorus uptake and utilisation of white clover (*Trifolium repens* L.)

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## Abstract

Excessive inputs of phosphorus have led to well documented problems with respect to water quality and the maintenance of critical ecosystem processes. Development of productive varieties of forage legumes with higher intrinsic phosphorus-use efficiencies (PUE) in terms of P acquisition, utilization and retention will result in lower fertiliser P requirements and P transfer to water courses by overland flow. In this experiment, clonal replicates of ninety-one genotypes of an IBERS white clover mapping family were grown in flowing solution culture for 28 days under low and high P with no added nitrate, to quantify variation in components of PUE. N<sub>2</sub> fixation was generally greater under high P than under low P. Variation in P uptake and utilisation efficiencies, but under low P increase in uptake-efficiency is associated with a larger incremental increase in utilisation efficiency than under high P supply.

Keywords: Trifolium repens, phosphorus, uptake, utilization

#### Introduction

Diffuse pollution of nitrogen (N) and phosphorus (P) from agricultural sources is high on the environmental protection agenda of many governments. Considerable effort is now going into developing strategies that will reduce the direct (leaching) and indirect (through animal returns) pollution and the environmental impact of grassland agriculture. Phosphorus is one of the most important determinants of both yield (Vance *et al.*, 2003) and environmental quality in agriculture systems; however, excessive inputs of phosphorus have led to well documented problems with respect to water quality and the maintenance of critical ecosystem processes. Development of productive varieties of forage legumes with higher intrinsic phosphorus use-efficiencies (PUE) within swards will result in lower fertiliser P requirements and P transfer to watercourses by overland flow. This paper reports progress of the IBERS forage legume breeding programme in quantifying variation in P acquisition, utilization and retention within white clover (*Trifolium repens* L.) as the first step in developing improved white clover varieties which are more efficient in their uptake and utilisation of phosphorus.

#### Materials and methods

Ninety-one genotypes of the IBERS white clover stem nematode mapping family (details are described in Febrer *et al.*, 2005) were grown in the IBERS flowing solution culture facility (FSC) over 28 days. Three clonal replicates of the 91 genotypes, derived from stolon cuttings, were selected for uniformity of size and placed in growth vessels within tanks supplied with an optimal (high) P concentration and with 1/5<sup>th</sup> strength P (low P). No N was supplied to the plants during the experiment, and therefore any N in plant tissue was produced by N-fixation only. After 28 days, the plants were harvested and yields (fresh and dry weight) of roots and shoots were measured. All herbage and root fractions were freeze dried prior to

grinding and analysis of total P and N to enable P uptake and utilisation efficiencies to be quantified. All data are presented as a mean of the three plants per growth vessel.

#### **Results and discussion**

The prerequisite for genetic improvement of nutrient-use efficiency is the existence of genetic variation in relevant physiological traits (Wilkins *et al.*, 1997). The aim of this experiment was to quantify the extent of genetic variation in response to different levels of P supply within white clover using genotypes from the IBERS stem nematode mapping family. Clonal replicates of the genotypes were used to enable direct comparison between uptake and utilisation efficiencies under different levels of P supply. In this study no nitrate was added to the flowing solution culture system. The N content of the white clover plants was therefore entirely the consequence of  $N_2$  fixation, which was generally greater under high P than under low P supply (Figure 1). The availability of P is important for  $N_2$  fixation; however, the data indicate significant genotypic variation for this trait. There was also significant genotypic variation in P uptake and utilisation efficiencies within the stem nematode mapping family, with strong linear relationships between uptake and utilisation efficiencies (Figure 2). Under low P supply, the increase in uptake efficiency (defined as mg per plant) was associated with a larger incremental increase in utilisation efficiency (defined as g tissue per g P) than under the high P supply. This suggests that selection for increased P



Figure 1.  $N_2$  fixation of plants under high or low P supply. Data are derived from 91 genotypes of a white clover mapping family grown in flowing solution culture over 28 days



Figure 2. P uptake (mg P plant<sup>-1</sup>) and P utilisation efficiencies (g tissue g<sup>-1</sup> P) of 91 genotypes of a white clover mapping family under high or low P supply grown in flowing solution culture over 28 days

utilisation efficiency under low P may also increase utilisation efficiency under high P supply. Further work is required to determine if selection for components of PUE in a flowing solution culture system will be reflected in increased PUE when plants are grown in the field.

#### Conclusions

Genetic variation in the main components of PUE, P uptake and P utilisation, were identified within the white clover stem nematode mapping family in an experiment in flowing solution culture. This information will be used to identify QTL for the components of PUE and transfer of QTL alleles into elite breeding material in the white clover breeding programme for further testing and validation in field trials on low P soils.

#### Acknowledgements

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## References

Febrer M., Jenkins G., Abberton M.T. and Milbourne D. (2005) Towards a comparative map of white clover (*Trifiolium repens*) and barrel medic (*Medicago truncatula*). In: Humphreys M.O. (ed.) Molecular Breeding for the Genetic Improvement of Forage Crops and Turf. A satellite workshop of the XXth International Grassland Congress, Wageningen Academic Publishers p. 193.

Vance C.P., Uhde-Stone C. and Allan D.L. (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a non-renewable resource. *New Phytologist* 157, 423–447.

Wilkins P.W., Macduff J.H., Raistrick N. and Collinson M. (1997) Varietal differences in perennial ryegrass for nitrogen use efficiency under simulated grazing in the field. *Euphytica* 98, 109–119.

# Norwegian output standards for N and P in livestock excreta

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## Abstract

In Norway the output standards of livestock excreta have not been updated in the last 15 years, and the scientific basis for the figures is even older. During that time there have been considerable changes in the intensity of Norwegian agricultural production. In this paper the prevailing Norwegian standards are presented and compared to output standards from Denmark, Great Britain, Sweden and Switzerland. There is a need for updated and improved output standards for N and P excretion for different types of farm livestock, probably in all Scandinavian countries. This can be achieved by different methods, which are discussed.

Keywords: livestock, excreta, slurry, nitrogen, phosphorus

## Introduction

Farm manures are a major source of nitrogen (N) and phosphorus (P) pollution. Excreta from livestock make a significant contribution to atmospheric ammonia emissions and losses of N and P from agricultural land. In order to reduce nutrient emissions from agriculture, it is important to have reliable N and P output standards for the major categories of farm livestock. The scientific basis for the Norwegian output standards of livestock excreta is more than 30 years old. In that time there have been considerable changes in agricultural production; e.g. the annual milk yield per cow has increased from about 5500 kg to 7200 kg.

## Materials and methods

In a report to the Norwegian Agricultural Authority, the Norwegian Institute for Agricultural and Environmental Research has presented the prevailing Norwegian output standards and the investigations which these standards are based upon (Nesheim *et al.*, 2011). More Norwegian references are given in the original report. Output standards from Denmark (Poulsen, 2009), Great Britain (Cottrill and Smith, 2010), Sweden (Jordbruksverket, 2010) and Switzerland (Flisch *et al.*, 2009) are also presented and discussed.

## **Results and discussion**

The output standard of excreted slurry per animal per year in Norway (Table 1) presented by Tveitnes *et al.* (1993), mainly originates from investigations carried out around 1975. The amounts of excreted nitrogen and phosphorus (Tveitnes *et al.*, 1993) are based on a combination of direct measurements and estimates from the middle of the 1980s, when the average milk yield was 5500 kg. The prevailing Norwegian output standards for dairy cows are 94 kg N and 14.8 kg P per year (Table 1), estimated in 1993 based on an annual milk yield of approximately 6200 kg (Bolstad, 1994). Volden (unpublished data) has by means of the planning tool "NorFor" estimated an N excretion of 96 kg at a milk yield of 6000 kg and of 108 kg N at a milk yield of 8000 kg. According to these figures, the annual N excretion should be 103 kg at 7200 kg milk, which is the current Norwegian average milk yield.
Some Danish output standards are presented in Table 2 (Poulsen, 2009). Two different breeds of dairy cows, Danish heavy race and Jersey, and two different weight classes of beef suckler cows are compared. The Swedish output standards are not presented. The Swedish figures for amounts of slurry are higher than shown in the other tables because litter, spill of water and rainfall are included in the figures. At a milk yield of 8000 kg the amount of slurry is 26.1 Mg and the excretion of nutrients is 117 kg N and 15.9 kg P. (Jordbruksverket, 2010). The corresponding figures for a yield of 10000 kg milk are 26.5, 139 and 17.4.

Table 1. Excretion of slurry (Mg year<sup>-1</sup>) and of nitrogen (N) and phosphorus (P) in kg animal<sup>-1</sup> year<sup>-1</sup> in Norway. Two different investigations in Norway (Tveitnes *et al.*, 1993; Bolstad, 1994). Litter, spill of water and rainfall are not included in the figures

	-				
Tune of settle	Tveitnes et	Bolstad	Bolstad (1994)		
Type of cattle	Slurry, Mg year <sup>-1</sup>	N, kg	P, kg	N, kg	P, kg
Dairy cow	18	82	12.6	94	14.8
Young cattle $> 12$ months	10	35	3.8	-	_
Young cattle 6–12 months	5	23	2.8	-	_
Heifers 0–12 months	-	_	_	27	2.2
Heifers 12–24 months	_	_	_	46	4.6
Bulls 0–12 months	_	_	_	20	3.3
Bulls 12–16,5 months	-	_	_	25	4.2

Table 2. Excretion of slurry (Mg year<sup>-1</sup>) and of nitrogen (N) and phosphorus (P) in kg animal<sup>-1</sup> year<sup>-1</sup> in Denmark (Poulsen, 2009). Litter, spill of water and rainfall are not included in the figures.

			-
Type of cattle	Slurry, Mg year <sup>-1</sup>	N, kg	P, kg
Dairy cow, heavy race, milk y. 9 239 kg year <sup>-1</sup>	21.3	130	20.9
Dairy cow, Jersey, milk yield 6 603 kg year <sup>-1</sup>	17.6	110	18.3
Young cattle, heavy race, 6-27 months	6.5	46	7.8
Young cattle, Jersey, 6–25 months	4.7	35	5.8
Beef suckler cow, 400-600 kg live weight	49	22	2.4
Beef suckler cow, $> 600$ kg live weight	5.5	25	2.7

Table 3. Excretion of slurry (Mg year<sup>-1</sup>) and of nitrogen (N) and phosphorus (P) in kg animal<sup>-1</sup> year<sup>-1</sup> in Switzerland (Flisch *et al.*, 2009). Litter, spill of water and rainfall are not included in the figures.

Type of cattle	Slurry, Mg year <sup>-1</sup>	N, kg	P, kg
Dairy cow, milk yield 6 500 kg year <sup>-1</sup>	23.0	115	18.0
Beef suckler cow, 600 kg live weight	15.5	80	13.0
Young cattle < 12 months	5.5	25	3.0
Young cattle 12–24 months	8.0	40	6.0
Young cattle $> 24$ months	11.0	55	9.0

Swiss figures for amounts of excreted slurry and nutrients for dairy cows, suckler cows and young cattle of different ages are shown in Table 3 (Flisch *et al.*, 2009). The English Advisory service organisation ADAS has developed 'Nitrogen production standards for livestock excreta' (Cottrill and Smith, 2010). The estimates are partly based on direct measurements, indirect calculations and modelling. At a milk yield of about 7 000 kg the excretion of slurry is 19.3 Mg and the output of N is 117 kg. For cows milking 9000 kg the excretion is 23.4 Mg slurry and 134 kg N. Laws *et al.* (2004) recorded all inputs and outputs of nitrogen on 86 British farms during one year. On average there were 126 dairy cows per farm and the average milk yield was 7185 litres. The average annual N excretion was 143 kg. The Norwegian output standards for livestock excreta are somewhat lower than the estimates from other countries, particularly for phosphorus. There is a need for updated and improved

output standards for N and P excretion for different types of farm livestock, probably in all Scandinavian countries. This can be achieved by different methods. At the Institute of Animal and Aquacultural Sciences at The Norwegian University of Life Sciences there is a current project on applying the tool "NorFor" to develop new standards for N and P excretion by different yield levels and forage regimes for dairy cows. There will also be a focus on excretion of N and P by pigs and poultry in the project. It would have been advantageous if investigations on improving the output standards for N and P excretion for different types of farm livestock could be accomplished as cooperative Scandinavian projects.

#### References

Bolstad T. (1994) Utskiljing av nitrogen og fosfor frå husdyr i Norge. Sluttrapport. Norges Landbrukshøgskole, Ås (In Norwegian).

Cottrill B. and Smith K.A. (2010) Nitrogen production standards for livestock excreta. Final report, Defra project WT0715NVZ. ADAS. 56 pp.

Flisch R., Sinaj S., Charles R. and Richner W. (eds) (2009) Grundlagen für die Düngung im Acker- und Futterbau, Chapter 11 'Hofdünger'. Forschungsanstalten Agroscope Changins-Waedenswil ACW und Agroscope Reckenholz-Tänikon ART. *AGRARForschung* 16(2), 50–61.

Jordbruksverket (2010) Statens Jordbruksverks författningssamling. Föreskrifter om ändring i Statens jordbruksverks föreskrifter och allmänna råd om miljöhänsyn i jordbruket vad avser växtnäring. SJVFS 2010, 55 (In Swedish).

Laws J., Smith K.A., Cottrill B. and Dewhurst R. (2004) Nitrogen and phosphorus excretion by UK dairy cows. *Proceedings of the 11th International Conference of the FAO ESCORENA Network on Recycling of Agricultural*, Municipal and Industrial Residues in Agriculture, RAMIRAN, Murcia Spain. I, pp. 35–38.

Nesheim L., Dønnem I. and Daugstad K. (2011) Mengd utskilt husdyrgjødsel – vurdering av normtal. *Bio-forsk Rapport* 6(74). 19 pp. (In Norwegian).

Poulsen H.D. (2009) Normtal for husdyrgødning – 2009. Det Jordbrugsvitenskapelige Fakultet, Århus Universitet. 35 pp. (In Danish).

Tveitnes S., Bruaset A., Bærug R. and Nesheim L. (1993) Husdyrgjødsel. Statens fagteneste for landbruket, Ås. 119 pp. (In Norwegian).

# The effects of the nitrification inhibitor dicyandiamide (DCD) on herbage nitrogen uptake when applied in late summer or early autumn

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#### Abstract

Dicyandiamide (DCD) has been shown to reduce nitrate leaching by slowing the conversion of ammonium to nitrate in the soil, thereby increasing the quantity of available nitrogen (N) in the soil for uptake by herbage. The objective of this experiment was to observe the effects of DCD on annual herbage N uptake when applied to the growing sward in late summer and early autumn. The experiment was a block design with three replicates of each treatment at two sites. The sites were: (1) a free-draining acid brown earth of sandy loam to loam in texture at Moorepark (MPK), and (2) a fine loam soil with imperfect drainage at Johnstown Castle (JC). The factors examined in the experiment were as follows: urine application, DCD rate and time of DCD application. Annual herbage N uptake increased significantly in both measurement years at MPK when urine was applied (P < 0.01). There was a significant effect (P < 0.01) of urine application date on annual herbage N uptake at both sites. There was no significant effect of DCD rate or application date on annual herbage N uptake at either site.

Keywords: herbage nitrogen uptake, nitrification inhibitor, dicyandiamide, urine patch

# Introduction

Urinary N has a high potential for leaching as the quantity of N excreted exceeds plant N requirements. Nitrification inhibitors such as dicyandiamide (DCD) have been shown to reduce nitrate (NO<sub>3</sub><sup>-</sup>) leaching and nitrous oxide emissions (Moir et al., 2007) by slowing the conversion of ammonium  $(NH_4^+)$  to  $NO_3^-$  in the soil, thereby increasing the quantity of N available for uptake by growing plants. Nitrate is readily taken up by growing plants, but if there is surplus available (e.g. under urine patches) it is likely to be lost through leaching. Urine patches are applied to approximately 25% of the surface area of a paddock over the duration of a year and provide a source of N to grazing swards (up to 1000 kg N ha<sup>-1</sup>) (Haynes and Williams, 1993). Di and Cameron (2002) showed that DCD applied to urine patches can potentially increase annual herbage production by up to 33%. Moir et al. (2007) reported that DCD significantly increased pasture N uptake from urine patches. Selbie et al. (2011) observed that DCD (10 kg ha<sup>-1</sup>) reduced NO<sub>2</sub><sup>-</sup> leaching by 45% and N<sub>2</sub>O emissions by 70% on dairy cow urine (1000 kg N ha<sup>-1</sup>) treatments on Irish soils. Temperature is the most influential environmental factor affecting the effectiveness of DCD; an increase in temperature can have a negative effect on the persistence of DCD in the soil. The half-life of DCD at 8°C has been shown to be 5 times longer than at 20°C, 111-116 days and 18-25 days, respectively (Di and Cameron, 2004). DCD applied in late summer at higher temperatures has the potential to degrade more rapidly than DCD applied in late autumn at lower temperatures. The objective of this experiment was to investigate the effect of DCD application on N uptake by herbage on two soil types at different times in late summer or early autumn.

# Materials and methods

The experiment was undertaken at the Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland (50°07'N, 08°16'W) (MPK), and at Johnstown Castle Research Centre, Teagasc, Co. Wexford, Ireland (52°18'N, 06°30' W) (JC) on two contrasting soil types. The soils were (1) a free-draining acid brown earth of sandy loam to loam texture at MPK, and (2) a fine loam soil with imperfect drainage at JC. The experimental area consisted of 13 simulated grazed plots (5 m  $\times$  1.5 m at MPK;  $5 \text{ m} \times 1 \text{ m}$  at JC) replicated three times in a randomised block design at each site. The experiment was a three factorial arrangement plus one control. Treatments were as follows: two urine rates (0 or 1000 kg N ha<sup>-1</sup>); three months of urine application (July, August, and September); two rates of DCD (0 or 10 kg ha<sup>-1</sup>) plus one control – a zero control receiving zero urine and zero DCD. The experiment commenced in July 2009 and was completed in April 2011. Simulated dairy cow urine (urea and water mix), deposited at a rate of 1000 kg N ha<sup>-1</sup> was used so that a known quantity of N was applied. This was applied as a single deposition using 10 litre watering cans with rose caps. Dicyandiamide was applied within 24 hours of simulated urine application as a Fine Particle Suspension (FPS) using a walk behind motor operated sprayer. Nitrogen fertiliser was applied using split applications between mid-January and mid-September as either urea (46% N; February to April) or calcium ammonium nitrate (CAN; 27% N; May to September). Herbage was harvested every 4 weeks from February to November using an Agria mower at MPK and using a lawnmower at JC. Cut herbage was weighed and a sub-sample (100 g) was dried at 40°C for 48 hours. The dried herbage was milled through a 1 mm screen. Spring herbage crude protein (CP) content was determined using near infra-red spectroscopy (NIRS) analysis. Spring herbage N uptake was computed using the following equations:

Herbage N content = herbage mass (kg DM ha<sup>-1</sup>) × herbage CP content (g kg<sup>-1</sup>) / ( $6.25 \times 1000$ )

Herbage N uptake = treatment herbage N content – control herbage N content

Data were analysed using PROC GLM in SAS. Data for each site were analysed separately.

# **Results and discussion**

There was no significant effect of DCD application rate on annual herbage N uptake at either site (Table 1). On average, urine application significantly (P < 0.01) increased annual herbage N uptake at MPK by 14% in year 1 and 44% in year 2 (Table 1). Applying urine in July significantly (P < 0.01) increased annual herbage N uptake in year 1 compared to August application of urine at MPK (Table 1). There was a significant (P < 0.01) effect of urine application date on annual herbage N uptake in year 2 compared to all other urine application dates at JC (Table 1). Plots showed signs of scorching at both sites following urine application. Middelkoop and Deenan (1990) reported that urine had a negative effect on herbage production in treated areas due to scorching; therefore affecting the herbage N uptake following urine application to treated plots. The increased herbage N uptake on the urine treatments at MPK maybe due to urea uptake rather than NO<sub>3</sub><sup>-</sup> uptake. There was no significant interaction between urine application date and DCD rate in both years at both sites.

Massurement Deried	M	PK	J	С
Measurement Period	Year 1	Year 2	Year 1	Year 2
Urine application				
Zero Urine	305	93	126	94
Urine	355	166	138	100
SEM	15.1	9	8.7	9.2
Significance	*	***	ns	ns
DCD rate (kg ha <sup>-1</sup> )			_	
0	338	137	129	107
10	333	138	136	92
SEM	17.0	14.5	8.8	8.9
Significance	ns	ns	ns	ns
Urine application date				
July	394	152	151	115
August	321	147	117	81
September	349	122	145	88
SEM	21.6	18.9	15.2	11
Significance	*	ns	ns	*

Table 1. The effects of DCD applied at rates of (0 and 10 kg ha<sup>-1</sup>) following urine applications of 0 or 1000 kg N ha<sup>-1</sup> in July, August or September on herbage N uptake (kg N ha<sup>-1</sup>) at Moorepark and Johnstown Castle

ns – not significant; \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001; SEM – standard error of the mean.

#### Conclusions

The application of DCD did not significantly affect herbage N uptake at either site during the experiment. The addition of urine increased herbage N uptake at MPK in both years; there was no effect at JC. Grass plots showed signs of scorching following the addition of urine.

#### References

Di H.J. and Cameron K.C. (2002) The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in simulated grazed and irrigated grassland. *Soil Use and Management* 18, 395–403.

Di H.J. and Cameron K.C. (2004) Effects of temperature and application rate of a nitrification inhibitor, dicyandiamide (DCD) on nitrification rate and microbial biomass in a grazed pasture soil. *Australian Journal of Soil Research* 42, 927–932.

Haynes R.J. and Williams P.H. (1993) Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Advances in Agronomy* 49, 119–199.

Middelkoop N. and Deenan P.J.A.G. (1990) The local influence of cattle dung and urine and its interactions with fertiliser nitrogen on herbage dry matter production. In: *Soil-grassland-animals relationships*, *Proceedings 13th General meeting of the European Grassland Federation*. Banska Bystrica, pp. 67–70.

Moir J.L, Cameron K.C. and Di H.J. (2007) Effects of the nitrification inhibitor dicyandiamide on soil mineral N, pasture yield, nutrient uptake and pasture quality in a grazed pasture system. *Soil Use and Management* 23, 111–120.

Selbie D.R., Lanigan G., Di H.J., Moir J.L., Cameron K.C. and Richards K.G. (2011) Improving nitrogen efficiency using a nitrification inhibitor on urine-affected soil – a grassland lysimeter study. In: *The Agricultural Research Forum 2011*, Tullamore, Co. Offaly, pp. 2.

# Nitrates in groundwater in areas occupied by grassland in Poland

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# Abstract

Based on monitoring studies conducted in 2008–2010, the nitrate pollution of groundwater occurring in grassland soil profiles was evaluated. It was found that in springtime, the nitrate concentrations in ground water across the country exceeded 50 mg dm<sup>-3</sup> in an average of 18.2% of the monitoring points, and concentrations were at the level of 25–50 mg dm<sup>-3</sup> for 16.4% of the monitoring points. In autumn, the percentage of monitoring points with contaminated ground waters, and threatened by nitrates in waters, was much smaller, and amounted to 10.1% and 11.1%, respectively. Most of the monitoring points with nitrate content in the class >50 mg dm<sup>-3</sup> occurred in the central provinces of the country: Mazowieckie, Kujawsko-Pomorskie, Wielkopolskie and Lodz. The content of nitrate-nitrogen in water samples varied depending on the kind of monitoring point, habitat, animal density, doses of mineral nitrogen fertilizers, manner of grassland usage and soil type.

Keywords: groundwater, grassland, nitrate

# Introduction

Grasslands play a very important role in protection of water quality, being a natural biological filter. This role of grassland has been confirmed in many experiments. However, investigations so far have been conducted at the scale of specially created research sites or on selected grasslands, and there is a lack of studies carried out at regional or national level. In Poland such a possibility was arose in 2008 by the creation of monitoring of nitrate in shallow ground waters in areas occupied by meadows and pastures. This monitoring was established by the National Agro-Chemical Station (KSCh-R) and regional agro-chemical stations in cooperation with the Institute of Technology and Life Sciences (ITP) in Falenty. Based on the results of this monitoring the answers to the questions are sought concerning the protective role of grasslands at the macro-scale, and also with reference to the current needs arising from the implementation of the Nitrates Directive in Poland. In this range, among other things, the following research problems are considered:

• what percentage of groundwater is contaminated by nitrates and threatened by them?

• how do agricultural practices and natural conditions affect nitrate concentration in groundwater? The aim of this study is to evaluate the nitrate pollution of shallow groundwater occurring in different meadow habitats in Poland, based on the results of that monitoring system, in terms of various natural and anthropogenic factors affecting this state.

# Materials and methods

Monitoring of groundwater quality in relation to nitrate in the areas occupied by grassland in Poland is conducted based on a specially established observational-investigative network. The scope of monitoring includes activities such as:

- preparation of characteristics of the monitoring points, to determine their specific features and the relationships with their surroundings (especially in terms of the impact of agricultural practices on water quality);
- water sampling and laboratory analysis.

Water samples for analysis from the test points are taken twice a year, in spring and autumn. In these samples nitrate concentrations are determined in the laboratory according to PN-EN ISO 13395 (2001). The above-mentioned works are led by the regional agrochemical stations. Obtained results are collected by KSCh-R.

The assessment of nitrate pollution of groundwater habitats of meadow and pasture is conducted at the ITP Falenty, based on data provided by KSCh-R. This assessment includes, in particular, such elements as:

- determination of the population of results within the following classes of water quality: 0–24.99, 25–39.99, 40–50 and >50 mg NO<sub>3</sub> dm<sup>-3</sup>, in accordance with the guidelines for reporting on its implementation of the Nitrates Directive by Member States (European Commission, 2008);
- determination of the influence of various factors of natural and agricultural origin on water pollution by nitrates.

#### **Results and discussion**

The groundwater monitoring network in the area of grassland in 2008 consisted of 388 monitoring points, in 2009 there were 398 points, and in 2010 there were 404 points. These points were represented by piezometers (in the greatest number), drainage wells and drain-pipe outlets. The average content of nitrate in groundwater in areas occupied by grassland in Poland in 2008–2010 amounted to 25.5–28.8 mg NO<sub>3</sub> dm<sup>-3</sup> in the spring time, and 17.5–20.4 mg NO<sub>3</sub> dm<sup>-3</sup> in autumn. The distribution of results relative to the average value was very large. In the spring of 2008, 2009 and 2010 the percentage of measurement points for which nitrate concentrations were found to be above the limit of 50 mg NO<sub>3</sub> dm<sup>-3</sup> (nitrate-polluted water in the understanding of Nitrates Directive) amounted to 20.6%, 18.3%, and 15.8%, respectively; and points for which levels were within the limits of 25–50 mg NO<sub>3</sub> dm<sup>-3</sup> (waters threatened by nitrates pollution in the meaning of the Nitrate Directive) were 16.5%, 16.6% and 16.1%. In the autumn, the percentage of monitoring points with waters polluted and threatened by pollution waters was lower than in spring time (Table 1).

Classes of nitrates concentration	The per	centage of	of monite	oring poin	nts in the	class,%	
Classes of infrates concentration,		spring			autumn		
$\operatorname{mg} \operatorname{NO}_3 \operatorname{dm}^3$	2008	2009	2010	2008	2009	2010	
0-24.99	60.8	61.8	63.9	72.4	70.9	67.8	
25-39.99	12.1	10.3	9.4	6.4	8.0	9.2	
40-50	4.4	6.3	6.7	1.8	3.8	4.0	
>50	20.6	18.3	15.8	8.5	9.8	12.1	
Lack of water in a monitoring point	2.1	3.3	4.2	10.8	7.5	6.9	

Table 1. Distribution of monitoring points	, depending on the nitrate content in water samples
in 2008–2010, and share of monitoring point	s, in which there was no water

In the spring of 2010, the number of monitoring points where the nitrate concentration in water exceeded 50 mg  $NO_3$  dm<sup>-3</sup> was lower than in the corresponding period in previous years. This was probably due to the different amounts of precipitation in the comparable period. Annual precipitation in Poland in 2010 amounted to 802.9 mm whereas in 2008 and 2009 it was 648.6 and 683.0 mm, respectively (GUS, 2010; 2011). The largest percentage of monitoring points with the content of nitrates in the class of >50 mg  $NO_3$  dm<sup>-3</sup> occurred in the central provinces such as Mazowieckie, Kujawsko-Pomorskie, Wielkopolskie and Lodz. The content of nitrate nitrogen in water samples varied, depending on: (i) the type of the monitoring point; (ii) category of agronomic soil; (iii) ruminant density on the farm (LU  $ha^{-1}$ ); and (iv) the dose of mineral nitrogen fertilizers (kg N  $ha^{-1}$ ). In this range we ascertained, particularly, that:

- Nitrate concentrations in water collected from drainage-wells and outlets of drain-pipes were generally of similar amounts, and were much larger than from piezometers, which may be associated with the fact that in the case of piezometers the water was taken from deeper levels.
- The lowest nitrate concentrations occurred in water samples from monitoring points located on heavy soils (in all analysed cases), whilst the largest concentrations were mainly in water samples from monitoring points located on light and very light soils. This is a typical situation in the case of heavy soils because they have little ability to water seepage into the soil profile, and the mineral components with it. The opposite situation occurs in the case of light and very light soils.
- In the middle of the analysed periods (spring 2009 and 2010, and autumn 2010), a directly proportional relationship appeared between the nitrate concentrations in water samples of water taken from monitoring points, and the density of grass-eating animals on the farm.
- Nitrate concentrations in water samples collected from the monitoring points were positively correlated with levels of mineral nitrogen fertilizers in each of the analysed periods (average applications of mineral nitrogen fertilizers in 2008, 2009 and 2010 were 68.2, 63.3 and 59.4 kg N ha<sup>-1</sup>, respectively). Based on the coefficient of linear correlation (R) designated to the significance level P = 0.01 (\*\*) and P = 0.05 (\*) there was a statistically significant dependence of this relationship. R values were: 0.2285\*\*, 0.1886\*\*, 0.1504\* and 0.2670\*\*, 0.2013\*\*, 0.1335\* in relation to the results obtained in springs of 2008, 2009, 2010, and in autumns of 2008, 2009, 2010, respectively.

#### Conclusions

The performed studies allowed the assessment of nitrate pollution of groundwater that occurred in the profile of grassland soils in Poland. Contamination was found to be significant. The largest share of waters polluted by nitrates (containing >50 mg NO<sub>3</sub> dm<sup>-3</sup>) was observed in the central part of Poland in areas with high stocking densities for pigs and cattle. The rates of mineral nitrogen fertilizer application used on areas occupied by grassland in Poland have a significant affect on the formation of nitrate concentrations in groundwater of these areas. The level of nitrate concentration was also linked to factors such as depth of water sampling (type of the monitoring point), soil type, livestock density in LU ha<sup>-1</sup> and the annual amount of precipitation (although there was no statistical significance of these relations). The results obtained may be useful in work on the revision of existing Nitrate Vulnerable Zone in Poland.

#### References

Commission (2008) 'Nitrates' directive 91/676/CEE. Status and trends of aquatic environment and agricultural practice". Development guide for Member States' reports. 29 pp. Available: http://circa.europa.eu/Public/ irc/env/wfd/library?l=/framework\_directive/nitrates\_directive/development\_final2008pdf\_2/\_EN\_1.0\_&a=d GUS (2010) *Maly rocznik statystyczny Polski 2010*. GUS: Warszawa, 724 pp.

GUS (2011) Mały rocznik statystyczny Polski 2011. GUS: Warszawa, 737 pp.

PN-EN ISO 13395 (2001) Water quality. Determination of nitrite and nitrate nitrogen, and their sum using flow analysis (CFA and FIA) with spectrophotometric detection.

# GIS-based analysis of spatio-temporal variation of climatological growing season for Austria

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#### Abstract

The temperature-driven length of growing season significantly influences management and productivity of grassland. In the past decades a trend of an earlier start of growing has been observed in many European regions, especially in the temperate zones. Climate experts expect an increase of this trend in the future due to global warming. A GIS model has been developed to determine start and end of growing season by using daily temperature surfaces in high spatial resolution. The data set for generating the temperature maps can be both observations in the past and climate scenarios. Several temperature threshold variations, according to state-of-the-art definitions of the climatological growing season, are processed and result in maps of information about start, end and length of the growing season. With long-term analysis of yearly results, spatial and temporal shifts can be identified and spatially visualized. It provides a data base to support an understanding of climate impact on local scaled changes of environmental conditions. Therefore, adaptation strategies do not have to be based purely on changing signals of temperature but also on grassland-relevant interpretation of climate data in growing season parameters.

Keywords: GIS, grassland productivity, spatial interpolation, temperature

# Introduction

The growing season can be defined as a period of time when basic environmental factors are suitable for growth. Plants in temperate zones are generally adapted to the seasonal cycle and are sensitive to temperature changes and to the length of photo period (Menzel, 2002). Analysis of spatio-temporal changes of the growing season needs long-term series of observations and their processing by a simple GIS model. The concept of climatological growing season based on temperature meets these requirements. Compared with grassland-specific phenological observations, temperature measurements at weather stations are available at a relatively high spatial density for most regions and over many years. Phenological events such as leaf unfolding in spring or leaf colouring in autumn are widely used as indicators to identify start and end of growing season (Linderholm, 2006). Timing of most phenological phases can be directly observed but also predicted by using temperature. The strong correlation between temperature and plant phenology, particularly in spring, allows a simplified definition of the climatological growing season based on temperature (Menzel, 2003). The proposed temperature-driven approach approximates grassland-specific phenological indicators of the growing season with a strong focus on their spatial implementation.

#### Materials and methods

Continuous temperature surfaces on a daily basis provide the background for the analysis of growing season by using temperature thresholds. The strong elevation dependency of temperature is the key factor for interpolation, especially for all applications on complex terrain. The implemented geostatistical algorithm refers to the state–of-the-art method *Residual Kriging*, which combines two different approaches (Goovaerts, 1997). Firstly, the monthly lapse rates are calculated by regression analysis of monthly mean temperature at each station and its elevation. To consider different lapse rates for lowland and highland mainly caused by inversions, the study region is separated according to both categories. Regressions are set up for each area and the resulting linear functions are applied on a Digital Elevation Model (DEM) with a spatial resolution of 250 meters. The elevation-dependent part of temperature is spatially interpolated by this altitudinal gradient-based first step. Secondly, the residuals of daily mean temperature are interpolated by Ordinary Kriging and added to the monthly surface of elevation-dependent mean temperature (Tveito, 2007). The different lapse rates of lowland and highland are smoothed at a small transition zone between the two areas which approximately reflects the elevation range of inversion height.

The start of growing season is assumed when daily mean temperature in spring exceeds a certain threshold for some consecutive days. A simple and widely used criterion found in literature is a threshold of 5°C and a period of five days (Frich *et al.*, 2002; Sparks *et al.*, 2005). We implemented this criterion as a raster algebra algorithm within ESRI ArcGIS programming environment. Five consecutive daily temperature surfaces are loaded and verified according to the proposed criterion. The results of this *Simple Thermal Definition (STD)* are stored in a raster dataset where each cell contains the date of start of growing season of the analysed year. The end of growing season is calculated by the inverted criterion: daily mean temperature drops below 5°C for at least five consecutive days.

STD with one single temperature-threshold is mainly used for station-based analysis but causes problems if spatial data with continuous temperature fields are taken into account. In some parts of the processed surfaces of growing season, unrealistic and misleading results have been found, particularly for extreme weather situations. Therefore, we extended the STD approach to a *Multiple Thermal Definition (MTD)* following the work of Brinkmann (1979) and Menzel *et al.* (2003). Different thresholds of daily mean and minimum temperature are combined to balance the sensibility of determination of start and end of growing season. The implemented raster algebra algorithm sets the start if mean temperature of a 10-day period exceeds 6°C and the criterion of STD is fulfilled on any five consecutive days within the 10 days. Additionally, a frost event within this period must not occur. This example of temperature-threshold combination defines a warm period in spring without harmful frosts. The end of growing season is processed in a similar way but with a focus on cold periods when growth slows down (Schaumberger, 2011). The temperature interpolation as the critical input for calculation of growing season was validated by leave-one-out cross validation.

#### **Results and discussion**

The estimated temperature at all Austrian weather station sites (total number of 270) was extracted from selected raster datasets and compared to the observations. A very strong correlation of 15,303 values of mean temperature was found with an R<sup>2</sup> of 0.98, a slope of 1.002, and a RMSE of 1.25°C. The statistics for minimum temperature are similar: an R<sup>2</sup> of 0.95, a slope of 1.000, and a RMSE of 1.72°C.

Raster surfaces of start, end and length of growing season according to STD and MTD have been calculated for the years 1971 to 2010 for the entire area of Austria at 250 meter resolution. Figure 1 shows the STD results as averages at all Austrian weather station sites. Trends of start and end are comparable to the findings of Menzel und Fabian (1999) who analysed long-term observations of phenological phases. The growing season starts earlier and ends later compared with the beginning of our study period, and results in an increase of duration with 3.2 days decade<sup>-1</sup>. MTD results on stations sites are very similar to STD results



Figure 1. Growing season (STD) trends for Austria over 40 years (average at all weather station sites)

but differ at the interpolated area between, especially in complex terrain. Realistic assignments of interpolated temperature to start or end of growing season require more than one single temperature-threshold.

# Conclusions

The climatological growing season does not consider the growth requirements of individual plant species, but gives a temperature-driven average with focus on climate. Long-term time series of start and end of growing season show changes along the timeline. To observe, visualize and evaluate changes also in their spatial dimension, a GIS-based analysis of growing season is needed and proposed by this work. In contrast to phenological observations, temperature values can also be derived from climate change models and processed in the same way as historical data to analyse future trends of growing season. Climate impact on grassland management can be estimated better – a pre-condition to work on efficient adaptation strategies.

#### References

Brinkmann W.A.R. (1979) Growing season length as an indicator of climatic variations? *Climatic Change* 2(2), 127–138.

Frich P., Alexander L.V., Della-Marta P., Gleason B., Haylock M., Klein Tank A.M.G. and Peterson T. (2002) Observed coherent changes in climatic extremes during the second half of the twentieth century. *Climate Research* 19, 193–212.

Goovaerts P. (1997) *Geostatistics for Natural Resources Evaluation. Applied Geostatistics Series*. Oxford University Press, New York, Oxford, 483 pp.

Linderholm H.W. (2006) Growing season changes in the last century. *Agricultural and Forest Meteorology* 137(1–2), 1–14.

Menzel A. (2002) Phenology: its importance to the Global Change Community. *Climatic Change* 54(4), 379–385.

Menzel A. (2003) Plant phenological anomalies in Germany and their relation to air temperature and NAO. *Climatic Change* 57(3), 243–263.

Menzel A. and Fabian P. (1999) Growing season extended in Europe. Nature 397(6721), 659-659.

Menzel A., Jakobi G., Ahas R., Scheifinger H. and Estrella N. (2003) Variations of the climatological growing season (1951–2000) in Germany compared with other countries. *International Journal of Climatology* 23(7), 793–812.

Schaumberger A. (2011) Räumliche Modelle zur Vegetations- und Ertragsdynamik im Wirtschaftsgrünland. Dissertation, Technische Universität Graz, Institut für Geoinformatik, 264 pp.

Sparks T.H., Croxton P., Collinson J.N. and Grisenthwaite D.A. (2005) The grass is greener (for longer). *Weather* 60(5), 121–125.

Tveito O.E. (2007) The developments in spatialization of meteorological and climatological elements. In Dobesch *et al.* (eds.) *Spatial Interpolation for Climate Data: The Use of GIS in Climatology and Meteorology.* ISTE Ltd., London, 73–86.

# Reducing N and K leaching from grassland: slurry applied by spreading or injecting?

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# Abstract

There is a need for fertilization practices that minimize nutrient leaching in Estonia, because average annual precipitation exceeds evaporation considerably. The objective of the study was to compare two slurry application methods, injecting and spreading, in terms of influence on the leaching of nitrogen (N) and potassium (K) from grassland. Mini-lysimeters filled with loamy sand soil embedded in three swards (three-species grass mixture, grasses mixture with white clover, and grasses mixture with lucerne) were used. Three annual nitrogen rates (60, 120, 180 kg ha<sup>-1</sup>) in conjunction with the two slurry application methods (injecting and spreading) were applied. Percolated water quantities, N and K content in leachate and the sward yield were measured. Leaching of N was significantly (P < 0.05) lower in the instance of slurry injection. K leaching was similar for both slurry application methods. Injection reduced N leaching only from grasses mixed with white clover sward. It can be concluded that slurry application method influenced only leaching of N. The botanical composition of the sward impacts the significance of application method influence on N leaching.

Keywords: application method, injecting, leaching, nutrient, slurry, spreading

# Introduction

Slurry is an organic and environmentally sustainable source of plant nutrition. Depending on the method of application, slurry remains either on the surface (spreading) or within the sward (injecting). The method of injecting slurry into the soil can efficiently prevent  $NH_3$  volatilization (Frost, 1994) and surface runoff losses of  $NH_4$ –N (Turtola and Kemppainen, 1998). However, large quantities of nutrients that are brought into the soil via slurry organic matter can mineralize when plants do not need them and can, therefore, lead to nutrient leaching (Bergström and Kirchmann, 1999).

The aim of the study was to examine whether injecting or spreading of slurry significantly influenced N and K leaching from grassland swards with different botanical composition.

# Materials and methods

The experiment was conducted from May 2008 to April 2010 at the Estonian University of Life Sciences (58°23'32" N, 26°41'31" E; elevation 60 m). Plastic mini-lysimeters (surface area 0.076 m<sup>-2</sup>; depth 30 cm) filled with loamy sand soil (sand 64%, silt 29%, clay 7%, specific surface area of  $30.6 \text{ m}^{-2} \text{ g}^{-1}$ ) were dug into the ground so that soil surface of the lysimeters were at the same level as the surrounding soil. At the beginning of the experiment the soil organic matter (OM) was 1.7-1.9%, total N content was 0.11%, available P and K (Egner–Rhiem–Domingo) was 94–102 mg kg<sup>-1</sup> and 165–180 mg kg<sup>-1</sup>, respectively. Precipitation from May 2008 to April 2009 was 709.6 mm, and from May 2009 to April 2010 was 743.8 mm.

The study involved the injecting or spreading of slurry equating to annual N rates of 60, 120 and 180 kg ha<sup>-1</sup> onto plots of three types of sward (i) grass mixture with *Phleum pratense*, *Lolium perenne* and *Poa pratensis*; (ii) grass mixture with added *Trifolium repens* and (iii) grass mixture with *Medicago sativa*. Slurry was applied manually. When injecting, holes (3 cm diameter, 5 cm depth) were made into the sward with a soil auger. The holes were filled with slurry and closed immediately with soil and a turf cap. Depending on the treatment, one to three separate applications of slurry were performed. Each application contained the proportional equivalent to 60 kg ha<sup>-1</sup> of nitrogen. The applications took place at the beginning of May, at the end of June, and at the beginning of August.

The quantities of leachate water and the total N and K content in the water were measured on a monthly basis throughout the vegetation period and after the vegetation period before the soil became frozen. The swards were harvested five times during the growing season. We used a VarioMax elemental analyser to measure the N content in the leachate and a flame photometer to measure the K content. To test the effect of sward type and slurry application method we used ANOVA and Fisher's LSD test to distinguish means. All calculations were carried out using the statistical package Statistica 9.0 (StatSoft Inc.) with the probability level set at 0.05.

#### Results

The average amounts of leached water percolated through 30 cm soil layer were 113.8 and 123.1 L m<sup>-2</sup> yr<sup>-1</sup> for the grass-white clover mixture, 153.5 and 164.7 L m<sup>-2</sup> yr<sup>-1</sup> for grass-lucerne mixture, and 158.3 and 164.1 L m<sup>-2</sup> yr<sup>-1</sup> for grasses, respectively, when slurry was injected or spread. Leaching of N was significantly (P < 0.05) less with slurry injecting. Slurry application method did not significantly influence K leaching. The quantities of N and K leaching correlated more to the botanical composition of the sward than on either method of slurry application. Slurry injecting reduced N leaching only from the grass-clover sward (Table 1). The highest leaching of N and K occurred in the grass sward, and the least amount was in the sward of grasses with white clover.

Harbaga	N 60 I	N 60 kg ha <sup>-1</sup>		kg ha <sup>-1</sup>	N 180 kg ha <sup>-1</sup>		
Tierbage	Spread	Injection	Spread	Injection	Spread	Injection	
		N g m	<sup>-2</sup> yr <sup>-1</sup>				
Grasses	3.13 <sup>aA1;2</sup>	3.17 <sup>aA</sup>	3.20.7 <sup>aB</sup>	2.89 <sup>aA</sup>	3.00 <sup>aA</sup>	2.75 <sup>aA</sup>	
Grasses + white clover	2.76 <sup>a1 B2</sup>	2.27 <sup>bB</sup>	$2.70^{\text{aAB}}$	2.12ыв	2.65 <sup>aA</sup>	2.08 <sup>bB</sup>	
Grasses + lucerne	2.94 <sup>aA</sup>	2.90 <sup>aA</sup>	$2.67^{aA}$	2.57 <sup>aA</sup>	3.19 <sup>aA</sup>	2.65 <sup>aA</sup>	
		K g m	<sup>-2</sup> yr <sup>-1</sup>				
Grasses	3.08 <sup>aA</sup>	3.08 <sup>aC</sup>	3.24 <sup>aC</sup>	2.95 <sup>aC</sup>	3.22 <sup>aC</sup>	2.26 <sup>bA</sup>	
Grasses + white clover	0.73 <sup>aB</sup>	1.01 <sup>aA</sup>	$0.76^{\mathrm{aA}}$	0.80 <sup>aA</sup>	0.97 <sup>aA</sup>	$0.94^{aB}$	
Grasses + lucerne	2.79 <sup>aA</sup>	2.19 <sup>aB</sup>	$1.87^{aB}$	1.92 <sup>aB</sup>	2.35 <sup>aB</sup>	2.00 <sup>aA</sup>	

Table 1. Average annual leaching of N and K according to the application method of slurry

<sup>1</sup>Within the same row, values with different lowercase letters within one N rate are significantly different (P < 0.05). <sup>2</sup>Within the same column, values with different capital letters are significantly different (P < 0.05).

Average sward yields were similar (P > 0.05) for both of the slurry-application methods, resulting in 735 g DM m<sup>-2</sup> and 773 g DM m<sup>-2</sup> for slurry spreading and injecting, respectively. The injection method in combination with higher N rates caused yields to increase more than with slurry spreading (Table 2). On all swards, the partial DM yields of grass species correlated most to a particular slurry application method (data not shown).

Table 2. Average DM yields depending on slurry application method

	-						
Harbooo	N 60 I	kg ha <sup>-1</sup>	N 120	kg ha <sup>-1</sup>	N 180 kg ha <sup>-1</sup>		
nerbage	Spread	Injection	Spread	Injection	Spread	Injection	
Grasses	484.1 <sup>aA1;2</sup>	535.9ªA	603.6 <sup>aA</sup>	669.4 <sup>aA</sup>	685.8 <sup>aA</sup>	832.9 <sup>aA</sup>	
Grasses + white clover	827.5 <sup>aB1</sup>	798.4ªB	864.3ªA	886.2ªA	$884.8^{aA}$	999.0ªA	
Grasses + lucerne	$610.7^{\text{aAB}}$	601.7 <sup>aAB</sup>	893.6 <sup>aA</sup>	767.5ªA	760.9 <sup>aA</sup>	862.8 <sup>aA</sup>	

<sup>1</sup>Within the same row, values with different lowercase letters within one N rate are significantly different (P < 0.05).

<sup>2</sup>Within the same column, values with different capital letters are significantly different (P < 0.05).

#### Discussion

A significant quantity of N volatilizes when slurry is spread (Frost, 1994), suggesting lower potential risk of N leaching. Our study did not confirm this hypothesis. Injection of slurry increased the availability of N to plants so it was used in yield formation. Our previous results (Raave *et al.*, 2010) demonstrate that leaching of N is positively correlated to the quantities of water percolating through the soil, which is in a negative correlation to the DM yield.

#### Conclusions

We conclude that the method of applying slurry has a significant influence only on N leaching, which was lower in slurry injection. Injection resulted in slightly higher DM yields and less percolation of water through the sward. Influence of slurry application method on N leaching depended on sward botanical composition and was significant only in sward grasses mixed with white clover.

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#### References

Bergström L.F. and Kirchmann H. (1999) Leaching of total nitrogen from 15N – labelled poultry manure and inorganic N fertiliser. *Journal of Environmental Quality* 28, 1283–1290.

Frost J.P. (1994) Effect of spreading method, application rate and dilution on ammonia volatilization from cattle slurry. *Grass and Forage Science* 49, 391–400.

Raave H., Kauer K., Noormets M., Selge A. and Viiralt R. (2010) Nitrogen and potassium leaching from grassland soil depending on applied fertilizer type and rate and sward botanical composition. *Grassland Science in Europe* 15, 1058–1060.

Turtola E. and Kemppainen E. (1998) Nitrogen and phosphorus losses in surface runoff and drainage water after application of slurry and mineral fertilizer to perennial grass ley. *Agricultural and Food Science in Finland* 7, 569–581.

# Total number of enchytraeids in the soil predicts the response of grasslands N yield to N fertilization on sandy soils

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# Abstract

Soil biotic parameters have seldom played a role in practical soil assessment and management of grasslands, so far. However, the ongoing reduction of external inputs in agriculture would imply an increasing reliance on ecosystem self-regulating processes. Since soil biota play an important role in these processes and in the provision of ecosystem services, soil biological parameters should be an integral part of soil assessment. The general objective of this study is to investigate to what extent soil biotic parameters provide additional value in soil quality assessment of grassland. In Experiment I, we measured in 2006 soil abiotic, biotic and process parameters together with N-yield and N-yield response in twenty permanent grasslands on sandy soils. The response of N yield to N fertilization ranged from 35–102%. This wide range underscores the importance of a better recommendation base to target N fertilizer. The response of N yield to N fertilization was predicted by the total number of enchytraeids in the soil. In 2010 this relation was checked in an identical experiment on three grasslands on sandy soil (Experiment II). Again a strong relation was found. This knowledge can be important for the optimal use of fertilizer and its consequences for environmental quality.

Keywords: grassland, soil quality, soil biota, apparent nitrogen recovery, enchytraeids, fertilizer recommendation

#### Introduction

Soil quality can be assessed by parameters based on chemical, physical and biological properties. However, biological soil properties have so far seldom played a role in practical soil assessment. The reduced use of external inputs implies a greater reliance on self-regulating processes (Brussaard *et al.*, 2007). Since soil biota play an important role in these processes and in the provision of ecosystem services, soil biological parameters should be an integral part of soil assessment. The general objective of this study is to investigate the extent to which biotic soil parameters have indicative and explanatory values in soil quality assessment of grassland on sandy soils. In Experiment I, conducted in 2006, abiotic and biotic soil parameters were measured in twenty grasslands with comparable management histories. In the growing season, grass yield at 0 kg N ha<sup>-1</sup> and response of grass yield to N fertilizer was measured. In Experiment II, conducted in 2010, this was repeated in three grasslands to check whether the results of 2006 could be repeated in time.

#### Materials and methods

#### Experiment I

This experiment was conducted in 2006, on twenty permanent grasslands on sandy soil distributed over ten conventional dairy farms. On each grassland, an experimental field (15 m  $\times$  9 m) was laid out. The first 10 m of the experimental field was split into three plots of 10 m  $\times$  3 m and the last 5 m in one plot of 5 m  $\times$  9 m. Over the three plots (10 m  $\times$  3 m), a fertilization treatment was randomized. Plots were fertilized with 0, 150

and 300 kg N ha<sup>-1</sup> yr<sup>-1</sup> with calcium ammonium nitrate (CAN, 27% N), respectively. The remaining 5 m  $\times$  9 m plot was not fertilized and was used to determine soil quality properties. All plots, except the 5 m  $\times$  9 m plot, received ample fertilization of P, K and S.

Soil samples were taken between 28 April and 2 May 2006 in the unfertilized 5 m  $\times$  9 m plot. In total 17 soil abiotic, 39 soil biotic and 17 soil process parameters were measured, including soil organic matter (SOM) and enchytraeids abundance ('potworms'). Grass was harvested four times using a 'Haldrup' small-plot harvester (J. Haldrup a/s, Løgstør, Denmark). Grass was weighed and sampled for dry matter and total N analysis (Kjeldahl). N yield as function of N level was modeled by a linear trend:

$$Y = \beta_{0i} + \beta_{1i} * Ngift + \varepsilon_{ij}$$

with the terms:

- Y N yield;
- $\beta_{0i}$  N yield intercept, or N yield of field *i* with 0 kg N ha<sup>-1</sup>;
- $\beta_{i}$  N yield response to N fertilizer; or the slope of the linear correlation between N yield and N application;
- $\varepsilon_{ii}$  random field effect,  $\varepsilon_{ii} \sim N(0, \sigma_V^2)$ .

Data analysis was performed with Matlab (version 7.6.0 R2008a, Mathworks). Crossvalidated stepwise regression was applied to find parameters that most accurately explained the N yield intercept (N yield at 0 kg N ha<sup>-1</sup>) and the slope of the N yield representing the response to N fertilization. More detailed information on sampling method and statistical analysis is referred to Van Eekeren *et al.* (2010).

#### Experiment II

This experiment was conducted in 2010 in three permanent grasslands on sandy soil distributed over one dairy farm. Plots were established and grassland production was measured using the same methods as in Experiment I. A selection of soil samples were taken in spring 2010 including determination of SOM and enchytraeids abundance.

# Results

#### Experiment I

N yield intercepts ranged from 78 kg to 263 kg N ha<sup>-1</sup>. The response of N yield to N fertilizer ranged from 0.35 to 1.02 kg N yield per kg N ha<sup>-1</sup> applied. SOM explained most of the variance in the N yield intercept and was selected in the stepwise regression as the best explanatory parameter for the N yield intercept ( $cvR^2 = 0.59$ , P = 0.001). One gram of SOM per kg dry soil meant 3.21 kg N yield ha<sup>-1</sup>, in addition to a constant of 15.4 kg N ha<sup>-1</sup>. In contrast to the intercept, the N fertilizer responses were not significantly correlated with the different abiotic soil parameters, except for a negative correlation with the C/N ratio in the soil. In the stepwise regression the response of N yield to N fertilization was significantly explained by the total number of enchytraeids ( $cvR^2 = 0.36$ , P = 0.047) (Figure 1).

# Experiment II

The N yield intercept of the three grasslands in Experiment II were 123, 141 and 293 kg N ha<sup>-1</sup> respectively. The response of N yield to N fertilizer was 0.56, 0.66 and 0.74 kg N yield per kg N ha<sup>-1</sup> applied. One gram of SOM per kg dry soil meant 3.39 kg N yield ha<sup>-1</sup>, in addition to a constant of 19.3 kg N ha<sup>-1</sup>. Like in experiment I the response of N yield to N fertilization was significantly explained by the total number of enchytraeids ( $R^2 = 0.94$ ) (Figure 1).



Figure 1. Linear regression between total number of enchytraeids (<sup>10</sup>log n m<sup>-2</sup>) and response of N yield to N fertilization (Exp. I:  $R^2 = 0.47$ , *cross validated*  $R^2 = 0.36$ , P = 0.047, Exp. II:  $R^2 = 0.94$ )

#### **Discussion and conclusions**

One of the main objectives of this study was to identify soil parameters in general, and soil biotic parameters specifically, that explain grassland production. It is interesting that variation of the response of N yield to N fertilization (0.35–1.02%) could not be explained by an abiotic soil parameter but only by a soil biotic parameter, the total number of enchytraeids. A possible explanation could be that enchytraeids indicate a nutrient-rich environment. A second explanation could be the effect of the historical grass production on the enchytraeids abundance measured at the start of both experiments. A third possible explanation is the important role of enchytraeids in decomposition processes. It is clear that the underlying mechanisms of this response need further investigation. This knowledge can be important for the optimal use of fertilizer and its consequences for environmental quality.

#### References

Brussaard L., de Ruiter P.C. and Brown G.G. (2007) Soil biodiversity for agricultural sustainability. *Agriculture, Ecosystems and Environment* 121, 233–244.

Van Eekeren N., de Boer H., Hanegraaf M., Bokhorst J., Nierop D., Bloem J., Schouten T., de Goede R. and Brussaard L. (2010) Ecosystem services in grassland associated with biotic and abiotic soil parameters. *Soil Biology and Biochemistry* 42, 1491–1504.

# Effect of harvest maturity of maize silage on dairy cow performance and enteric methane emission estimated using the Dutch Tier 3 approach

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# Abstract

A study was conducted to evaluate the effect of harvest maturity of maize silage (maturity 300, 340, 380 and 420 g DM kg<sup>-1</sup>) on dry matter intake (DMI), milk performance and *insitu* rumen digestibility. Enteric  $CH_4$  emissions were estimated according to the Dutch IPCC Tier 3 approach. Increasing harvest maturity of maize silage resulted in increased starch content and the proportion of rumen by-pass starch. However, there were no significant effects on feed intake and milk production. Model calculations indicated a small reduction of methane production per unit of DMI with increased harvest maturity.

Keywords: maize silage, harvest maturity, enteric CH<sub>4</sub> emission, dairy cow

# Introduction

Enteric methane (CH<sub>4</sub>) emissions from Dutch dairy farms can potentially be reduced by 2.1 Mt of  $CO_2$  equivalents by increasing starch and rumen by-pass starch in dairy cow rations (Tamminga *et al.*, 2007). Increased harvest maturity (HM) of maize silage results in an increased concentration of starch and by-pass starch. Therefore, increasing HM could be a simple and low-cost measure to increase dietary starch, thereby reducing CH<sub>4</sub> emissions from dairy farms. However, farmers are reluctant to apply this measure because of uncertainties about the effects of increased HM of maize silage on dry matter (DM) intake and subsequent milk yield. Therefore, an experiment was conducted to evaluate the effects of harvest maturity of maize silage on feed intake, milk yield and milk composition, and *in-situ* degradability. Subsequently, enteric CH<sub>4</sub> emissions were estimated according to the Dutch IPCC Tier 3 approach for dairy cows, based on a dynamic, mechanistic model described by Bannink *et al.* (2011).

# Materials and methods

Four silages were prepared from a single maize crop (cv. Atrium; Force Limagrain), on a marine clay soil (52°5'N, 5°5'E), fertilized with 230 kg N and 95 kg P2O5 ha-1 from cattle slurry and NP fertilizer. The maize was harvested (theoretical chop size 6 mm with kernel processing) at four stages of HM: 300 (M30), 340 (M34), 380 (M38) and 420 (M42) g DM kg<sup>-1</sup>, and stored in bunker silos. The maize silages were utilized in a 14-week lactation trial, using 64 Holstein-Friesian dairy cows, in a randomized complete block design. The cows were grouped in blocks of eight on the basis of similarity in parity, calving date, and milk performance during the previous lactation. The cows of each block were assigned to one of 8 dietary treatments which were a factorial combination of four maize silages (MS30, MS34, MS38 and MS42) and two types of concentrate, LC and HC. Concentrates LC and HC were iso-energetic and iso-nitrogenous but differed in concentration of water soluble carbohydrates (WSC) (Table 1). The maize silages were offered ad libitum as part of a forage mixture, containing 61% maize silage, 28% grass silage, 10% soybean meal and 1% minerals, on a DM basis. Each cow received 8.5 kg DM of concentrate. All feeds were analysed for DM, CP, OM, starch, sugars, NDF and in vitro digestibility of organic matter (OMD) (Table 1). Net energy for lactation (NE<sub>1</sub>) and intestinal digestible protein (DVE) were calculated according to CVB (2007). Dry matter intake (DMI), milk yield and body weight of individual cows was recorded daily throughout the experiment. Milk samples were taken weekly from four consecutive milkings and analysed for fat and protein concentration. From each maize silage, fifty nylon bags were ruminally incubated in three fistulated cows according to CVB protocol (2003). The maize silage samples were unground, except for particles with a size larger than 1 cm which were cut manually. After incubation, the nylon bags were pooled by incubation time and analysed for DM, ash, starch, NDF and N concentration. The data were fitted on the model of Ørskov and McDonald (1979):  $Y(t) = W+D\times(1-e^{kd\times t})$  in which the fraction W is the washout fraction which disappeared from the 0 h nylon bag after machine washing with tap water for 45 min., the D fraction is calculated as 1-W-U, in which U is the undegraded fraction after 336 h rumen incubation, and kd is the fractional degradation rate (h<sup>-1</sup>). The proportion of rumen by-pass starch (B) was calculated as (kp/(kp+kd)×(1–D)+0.1×S, with kp being the fractional rumen passage rate (h<sup>-1</sup>) and the under assumption that 10% of the soluble starch is rumen by-pass starch. The degradation characteristics determined by *in-situ* incubation of nylon bags were used as model inputs to estimate enteric CH<sub>4</sub> emission by the Tier 3 approach.

Table 1. Chemical composition, *in-vitro* digestibility and Net Energy ( $NE_L$ ) content of the forages and concentrates fed to lactating cows (values g kg<sup>-1</sup> DM, unless indicated otherwise)

	Maize silages				Grass	Soybean	Conce	ntrates
	MS30	MS34	MŠ38	MS42	silage	meal	LC	HC
DM at harvest (g kg <sup>-1</sup> )	296	341	396	421	NĎ	ND	ND	ND
DM silage (g kg <sup>-1</sup> )	341	354	376	410	286	879	897	897
Crude protein	74	77	79	78	136	385	207	196
WSC <sup>1</sup>	4.2	4.3	4.0	4.0	58	113	90	119
Starch	381	396	415	433	_	23	94	86
NDF	366	350	345	341	487	146	386	312
$OMD^2$ (%)	75.9	76.1	75.7	75.6	77.7	89.9	82.1	84.3
$NE_{L}^{3}$ (MJ kg <sup>-1</sup> DM)	6.6	6.6	6.5	6.5	6.3	8.0	7.3	7.3

<sup>1</sup>Water soluble carbohydrates; <sup>2</sup> in vitro organic matter digestibility; <sup>3</sup>Net energy for lactation.

The effects of HM, concentrate and stage of lactation on DMI and nutrient intake, milk yield, milk composition and body weight were determined by repeated measurement analysis of variance using the PROC MIXED procedure of SAS® (2003). Weeks of lactation were considered as a repeated effect on individual cows. The HM of maize silage, concentrate type and stage of lactation were fixed effects and block was considered as a random effect. Interactions between HM and type of concentrate were non-significant and therefore excluded from the model. The model used is described by  $Y_{ijkl} = \mu + M_i + C_j + W_k + e_{ijkl}$ , with  $Y_{ijkl}$  the dependent variable,  $\mu$  the general mean,  $M_i$  the fixed effect of HM (i = MS30, MS34, MS38 and MS42),  $C_j$  the fixed effect concentrate types (j = HC and LC),  $W_k$  the fixed effect of the repeated measurements of experimental weeks (k = 1–14), and  $e_{ijkl}$  the residual error.

#### Results

Increased HM resulted in an increased intake of starch, and reduced intake of NDF, but there were no effects on DMI and NE<sub>L</sub> intake. The cows fed the HC concentrate had higher WSC intake and lower NDF and CP intake. Milk fat yield was reduced in cows fed MS42, milk and protein yields were improved for the LC treatment. There were no effects of HM or concentrate type on the yield of FPCM (Table 2). The *in-situ* nylon bag degradation indicated a reduced proportion of starch digested in the rumen and an increased outflow of rumen by-pass with increasing HM. Increased HM was associated with a small reduction of estimated CH<sub>4</sub> conversion factor (% GE intake) and CH<sub>4</sub> emission per day or per kg DM (g d<sup>-1</sup>) (Table 3).

	-										
Demonstern	Harvest			Harvest maturity maize silage <sup>1</sup>						gnifica	nce <sup>3</sup>
Parameter	MS30	MS34	MS38	MS42	$SEM^4$	LC	HC	SEM	MS	Con	Week
Maize silage DMI	9.7	10.0	10.0	9.5	0.38	9.9	9.7	0.35	ns	ns	***
Total DMI	23.2	23.5	23.7	22.8	0.68	23.4	23.1	0.64	ns	ns	***
Crude Protein	3.40	3.47	3.53	3.40	0.09	3.52	3.38	0.09	#	***	***
Starch	4.42 <sup>a</sup>	4.71 <sup>b</sup>	4.89°	4.89°	0.16	4.68	4.68	0.15	**	ns	***
WSC <sup>5</sup>	1.27	1.27	1.29	1.24	0.03	1.16	1.38	0.03	#	***	***
NDF <sup>6</sup>	$8.58^{a}$	8.45 <sup>a</sup>	8.39 <sup>a</sup>	8.06 <sup>b</sup>	0.24	8.72	8.02	0.23	*	***	***
NE <sup>7</sup>	160	162	159	156	4.4	161	159	4.1	ns	ns	***
$DVE^8$	1.95	1.99	1.97	1.93	0.05	1.97	1.95	0.05	ns	ns	***
Milk	40.2	40.8	40.8	39.5	1.32	41.3ª	39.3 <sup>b</sup>	0.94	ns	*	***
Milk Fat	1.66 <sup>ab</sup>	1.70 <sup>a</sup>	1.70 <sup>a</sup>	1.60 <sup>b</sup>	0.07	1.67	1.67	0.05	**	ns	***
Protein	1.31	1.33	1.31	1.27	1.31	1.33ª	1.28 <sup>b</sup>	0.06	ns	*	***
FPCM <sup>8</sup>	42.9	43.4	43.8	41.6	1.45	42.9	43.4	1.02	ns	ns	***
Body weight (kg)	626	640	652	650	16.3	638	646	11.5	ns	ns	ns

Table 2. Effect of maize silages (MS) ensiled at different harvest maturities in combination with a low (LC) or high (HC) degradable carbohydrate concentrate (Con) on cow performance (dry matter intake (DMI), nutrient intake, milk yield)

<sup>1</sup>Dry matter contents of 300 (MS30), 340 (MS34), 380 (MS38) and 420 (MS42) g kg<sup>-1</sup> fresh weight; <sup>2</sup>LC low water soluble carbohydrates (WSC), HC high WCS; <sup>3</sup>ns, not significant; #P < 0.1; \*P < 0.05; \*\*P < 0.001; \*\*\*P < 0.001; <sup>4</sup>Standard error of the mean; <sup>5</sup>Water soluble carbohydrates; <sup>6</sup>Net energy for lactation; <sup>7</sup>Intestinal digestible protein, <sup>8</sup>Milk yield corrected to 4% and 3.32% protein, 1 FPCM corresponds with 3.05 MJ NE<sub>4</sub>.

Table 3. Results of the in-situ starch digestibility and estimation of enteric methane (CH4) production using the Dutch TIER 3 approach (Bannink *et al.*, 2011)

		HM mai	Concentrate <sup>2</sup>			
_	M30	M34	M38	M42	LC	HC
RD <sup>3</sup> Starch (%)	66.3	67.5	65.9	61.7	64.7	65.3
Starch outflow (%)	33.7	32.5	34.1	38.3	35.3	34.7
$GEI^4$ (MJ d <sup>-1</sup> )	420	419	420	421	422	418
MCF % of GEI	5.64	5.67	5.56	5.53	5.54	5.66
$CH_{4}g d^{-1}$	425	425	423	418	427	428
$CH_4^4$ g kg $DM^{-1.5}$	18.3	18.5	18.4	18.2	18.3	18.5

<sup>1</sup>Dry matter contents of 300 (MS30), 340 (MS34), 380 (MS38) and 420 (MS42) g kg<sup>-1</sup> fresh weight; <sup>2</sup>LC low water soluble carbohydrates (WSC), HC high WCS; <sup>3</sup>Rumen digested starch <sup>4</sup>Gross energy intake, <sup>5</sup>Methane Conversion Factor, <sup>5</sup>Assuming a DMI of 23 kg DM d<sup>-1</sup>.

#### Conclusions

Increased HM of maize silage has no adverse effect on DMI and NE<sub>L</sub> intake, or on milk and milk protein yield. Although fat yield was reduced at a HM of 420 g DM kg<sup>-1</sup>, FPCM yield was not affected. Increased HM of maize silage was accompanied by an increased concentration of starch and by-pass starch in maize silage, increased rumen by-pass starch, and a small reduction of methane production per unit of DMI. Increasing HM might be a simple and low-cost measure to reduce CH<sub>4</sub> emissions from dairy farms without compromising cow performance. *In vivo* data in combination with accurate methane measurements are preferred to evaluate this finding *in vivo*.

#### References

Bannink A., van Schijndel M.W. and Dijkstra J. (2011) A model of enteric fermentation in dairy cows to estimate methane emission for the Dutch National Inventory Report using the IPCC Tier 3 Approach. *Animal Feed Science and Technology* 166–167, 603–618.

CVB (2003) Protocol voor in situ pensincubatie – Bepaling van afbraaksnelheid en uitwasbare fracties van eiwit,zetmeel, celwanden en organische restfractie. Centraal Veevoederbureau, Lelystad, Nederland, pp. 14. CVB (2007) Table Ruminants, chemical composition and nutritional values of feedstuffs and feeding standards, Series no 32. Central Bureau for livestock feeding (CVB), the Hague, the Netherlands.

Ørskov E.R. and McDonald I. (1979) Estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science* 92, 499–503.

Tamminga S., Bannink A. and Zom R.L.G. (2007) Feeding strategies to reduce methane loss in cattle Wageningen UR Animal Sciences Group, Lelystad, Rapport 34 pp. 4.

# Biomass yield and carbon sequestration of energy grasses

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# Abstract

There are many 'grassland goods and services' such as food for livestock, biodiversity, tourism and recreation as well as biofuels and carbon storage. These two last 'goods' are of potential high importance for global  $CO_2$  reduction. Biomass yield and C-sequestration of three grass species: *Miscanthus* x giganteus Gref et Deu., *Elytrigia elongata* (Host) Nevski and *Phalaris arundinacea* L. were estimated on the basis of cultivation in different fertilization regimes: NPK 0-0-0, 80-20-30 (low dose) and 160–20–30 (high dose). Highest biomass yield (3-years average of 24.4 t of dry matter per ha) was obtained for *Miscanthus* fertilized under high dose; however, other species yielded high only at lower dose. It has been also concluded that after 33 months of cultivation average carbon increase in soil was ca. 2.32 t C ha<sup>-1</sup>. Species of the highest potential for C-sequestration were *Phalaris arundinacea* and *Miscanthus* x giganteus especially when fertilized.

Keywords: Miscanthus, Phalaris, Elytrigia elongata, renewable energy, carbon sink

# Introduction

Although there are issues surrounding the use of food crops (1<sup>st</sup> generation) for energy (the food vs. fuel debate), much research effort is currently being put into 2<sup>nd</sup> generation (non-food crops) and 3<sup>rd</sup> generation (dedicated bioenergy crops), which have better energy balances and do not compete for land with food production. Dedicated bioenergy crops are the link between sink (biomass/soil organic carbon) and the source (fossil fuel combustion). They are the sink/source transition, since the C incorporated in their biomass and roots has a high potential for being incorporated into existing soil organic carbon pools. The rate at which C accumulates in the soil varies with plant species and biomass productivity, site history, management practices and properties of the soil (Lemus and Lal, 2005). We therefore decided to investigate C-sequestration in three perennial energy grasses after three years of cultivation with different fertilization regimes.

# Materials and methods

Three perennial grass species: *Miscanthus* x *giganteus*, *Elytrigia elongata* and *Phalaris arundinacea* were used in our experiment. To produce new planting material of *Miscanthus*, three-year-old plants were split while dormant (end of April, 2008), using a rotavator, and the rhizome pieces were collected for re-planting. Planting density was 1 plant per 1 m<sup>-2</sup>. Seed of *Elytrigia* was obtained from our own reproduction of Bamar strain, and seeds of the *Phalaris* cultivar Keszthelyi were kindly provided by the breeder. Sowing quantities were, respectively, 15 kg ha<sup>-1</sup> and 11 kg ha<sup>-1</sup>. Both species were sown in May 2008. Before sowing and planting, soil samples were taken at 0–30 cm depth for the analysis of soil organic carbon (SOC) content. Since spring 2009 three different levels of fertilization were applied to each species each year: control (no fertilization), low level (80–20–30 of N-P-K) and high level (160–20–30 of N-P-K). During February – March each year, above-ground biomass was cut off (10 plots of 1m<sup>2</sup> of each species and each fertilization level) and yield was measured. In spring 2011, soil samples were taken from each species and each fertilization level for chemical analysis. Chemical analyses were performed at Regional Agro-Chemical Station. Statistical calculations were made according to SAS ® statistical package (SAS, 2004a; b).

#### **Results and discussion**

Fertilization significantly increased yield of all tested species (Table 1). The highest yield was obtained for *Miscanthus* fertilized at high level (160–20–30 of N-P-K). In the no-fertilized treatment, biomass yield was reduced (compared to optimal yield level) by 36% in the case of *Phalaris* and *Elytrigia*, and 30% for *Miscanthus*.

After 4 years of cultivation only minor changes in C contents were observed (Table 2). No differences were found between species or fertilization levels but only between initial value (2008) and average value of all species in 2011.

		Level of fertilization (N-P-K)					
Genus, species	0-0-0	80-20-30	160-20-30	(P = 95%)			
	(control)	(lower level)	(higher level)	(I - JJ/0)			
Phalaris arundinacea	6.1	9.5	9.9	4.1			
Elytrigia elongata	6.6	10.4	9.5	2.5			
Miscanthus x giganteus	17.2	19.7	24.4	5.6			

Table 1. Mean biomass yield (t ha-1 DM) from 3 harvests (2008/09, 2009/10, 2010/11)

3.7			SOC		
Year	Genus, species	Fertilization levels (N-P-K)	(%)		
2008	initial value - before cultivation		0.402		
2011	Elytrigia elongata	0-0-0	0.425		
		80-20-30	0.414		
		160-20-30	0.433		
	significance of difference betwee	n 2008 and 2011	ns		
	Phalaris arundinacea	0-0-0	0.456		
		80-20-30	0.485		
2011		160-20-30	0.480		
	significance of difference betwee	n 2008 and 2011	ns		
	Miscanthus x giganteus	0-0-0	0.394		
		80-20-30	0.487		
		160-20-30	0.507		
	significance of difference betwee	n 2008 and 2011	ns		
Significance of difference between:					
species		ns			
fertilizatio		ns			
years (2008 vs. 2011 average) **					

Table 2. Content of Soil Organic Carbon in the tested species under different fertilization levels

Assuming thickness of topsoil is 30 cm and soil bulk density is 1.5 g cm<sup>-3</sup>, it is possible to estimate the average annual organic C increase from -0.12 t C ha<sup>-1</sup> (*Miscanthus*, no fertilization) to + 1.17 t C ha<sup>-1</sup> (*Miscanthus*, high level of fertilization) (Figure 1).

Perennial grasses add soil organic matter by pre-harvest losses and harvesting residues (El Bassam, 2010). Considering estimations made so far it is possible to sequester more than 0.6 t C ha<sup>-1</sup> year<sup>-1</sup> in perennial grasses grown for bioenergy in monocultures (Smith *et al.*, 2000; Conant *et al.*, 2001; Lemus and Lal, 2005; Clifton-Brown *et al.*, 2007). Such data are close to our results for *Phalaris* and fertilized *Miscanthus*. High sequestration values could be noted also in the case of mixed cultivation (Tilman *et al.*, 2006).

The above results showed that adoption of bioenergy crops as perennial grasses could reduce the use of fossil fuels and offset other fuels by converting atmospheric  $CO_2$  into unharvested biomass residues and SOC (Lemus and Lal, 2005). Perennial grasses may be more suitable for C sequestration than willow, since the latter takes more time for canopy closure, making soil more prone to SOC losses (Harmon *et al.*, 1990).



Figure 1. Estimated C – sequestration in three grass species (vertical axis – species and fertilization levels)

#### Conclusions

Perennial grass cultivation during 33 months from establishment (sowing or planting) increased the soil organic carbon content by 2.32 t C per ha on average. Grass species of the highest carbon sequestration were *Phalaris arundinacea* and *Miscanthus giganteus*, especially when fertilized.

# References

Clifton-Brown J.C., Breuer J. and Jones M.B. (2007) Carbon mitigation by the energy crop, *Miscanthus*. *Global Change Biology* 13(11), 2296–2307.

Conant R.T., Paustian K. and Elliott E.T. (2001) Grassland management and conversion into grassland: effects on soil carbon. *Ecological Applications* 11(2), 343–355.

El Bassam N. (2010) Handbook of Bioenergy Crops. Earthscan Ltd., London, UK, pp. 516.

Harmon M.E., Ferrell W.K. and Franklin J.F. (1990) Effects of carbon storage of conversion of oldgrown forests to young forests. *Science* 247, 699–702.

Lal R. (2003) Global potential of soil carbon sequestration to mitigate the greenhouse effect. *Critical Reviews in Plant Science* 22(2), 151–184.

Lemus R. and Lal R. (2005) Bioenergy crops and carbon sequestration. *Critical Reviews in Plant Science* 24, 1–25.

SAS Institute Inc. (2004a). SAS 9.1 Companion for Windows. Cary, NC, USA, SAS Publishing, SAS Institute Inc.

SAS Institute Inc. (2004b). SAS/STAT 9.1 User's Guide. Cary, NC, USA, SAS Publishing, SAS Institute Inc. Schlezinger W.H. (1999) Carbon sequestration in soils. *Science* 284, 2095.

Smith P., Powlson D.S. and Smith J.U. (2000) Meeting Europe's climate change commitments: quantitative estimates of the potential for carbon mitigation by agriculture. *Global Change Biology* 6, 525–539.

Tilman D., Hill J. and Lehman C. (2006) Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science* 314, 598–600.

# Relations between bioclimatic variables and endophyte colonization of grasses in Poland

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# Abstract

The occurrence of endophytes in wild grasses in Poland was analysed in 2007–2009. The results suggest that endophytes are common in Poland and colonize ca. 70% of visited localities, mostly semi-natural grasslands. It is shown that the presence of endophytes in a given location is positively related to thermal conditions and negatively to precipitation. Accordingly, endophytes were mostly found on grasses growing in relatively dry regions with high temperatures, especially in summer.

Keywords: grassland, climate, Neotyphodium, Festuca pratensis, Festuca arundinacea, Festuca rubra

# Introduction

Endophytes are symptomless fungi from *Neotyphodium* species (Siegel et al., 1985; White and Cole, 1985; Gams et al., 1990; Wilson, 1995). The mycelium of the endophytic fungi grows in intercellular spaces of leaf sheaths, leaf blades, culms, floral organs and rhizomes. The host plant flowers and sets viable seed, spreading endophyte vertically through the seed. From an agronomic standpoint the grass-Neotypodium symbiosis can have positive and negative effects. Grasses colonized by *Neotyphodium* (E+) express adaptations to abiotic stress (drought, mineral imbalance, soil acidity) and biotic stress (disease, pests or herbivores). In certain circumstances the endophytic fungus may produce toxic alkaloids (ergovaline, lolitrem B, etc.) which play a role in defending the host plant against herbivores. Hence, the presence of *Neotyphodium* has been linked with decreased animal production and health problems. Current knowledge on the nature of major forces driving endophyte presence and infection frequency in semi-natural permanent grassland is very limited. Among the many biotic and abiotic factors that determine plant performance in natural conditions (water and light, soil, nutrients, organic matter, herbivore activity, pests and diseases, etc.) climatic conditions are of the most 'uncontrolled' nature. Possibly climate change will influence the Neotyphodium - grass symbiosis (Rudgers and Swafford, 2009). The aim of our study was to describe effect of bioclimatic variables on endophyte occurrence in permanent grasslands in Poland.

#### Materials and methods

During 2007–2009 more than 220 permanent grasslands in Poland were screened for the presence of endophyte fungi on grasses. The material consisted of plants of 26 grass species collected in 266 locations in Poland (645 ecotypes), especially *Festuca rubra* L., *F. pratensis* Huds., *F. ovina* L., *F. arundinacea* Schreb., *F. capillata* Lam., *Lolium perenne* L., *L. multiflorum* Lam., *Deschampsia caespitosa* (L.) P.B., *D. flexuosa* (L.) Trin. and *Poa pratensis* L. All collected plants were checked for the presence of the genus *Neotyphodium*. The staining method with rose bengal by Saha *et al.* (1988) was used for the detection of fungi. For each location, GPS coordinates were noted during collection. For each collection site endophyte presence was expressed as: 0 – no endophyte, 1 – endophyte in at least one

ecotype per site. Further, using DIVA-GIS ver. 7.1.7 software (http://www.diva-gis.org) 21 bioclimatic variables were ascribed to each collection site (for details see Table 1). Statistical analysis (logistic regression) was performed using SAS® statistical package.

#### **Results and discussion**

More than 713 grass ecotypes from 26 species were analysed. Endophytic fungi were found in the majority (69%) of the 226 visited localities (Figure 1).



Figure 1. Distribution of collection site with endophytes (solid circles) and without endophytes (open circles)

Average endophyte presence in collected ecotypes was 31%, but included only 10 species, mostly from the *Festuca* and *Lolium* genera. Ecotypes from the mentioned genera were inhabited by endophytes from 14% for *F. capillata* to 71% for *F. pratensis* (mean 45% for *Festuca* species) and from 25% for *L. multiflorum* to 29% for *L. perenne* (mean 29% for the *Lolium* species). The following species were completely free from endophytes: *Agrostis gigantea, Alopecurus pratensis, Arrhenatherum elatius, Bromus inermis, Cynosurus cristatus, Danthonia decumbens, Deschampsia flexuosa, Festuca gigantea, F. heterophylla, Glyceris fluitans, Holcus sp., Koeleria macrantha, Phleum boehmerii, Poa compressa, P. nemoralis, P. palustris. Similar results were obtained by Wäli <i>et al.* (2000), considering populations of wild grasses in Finland. The highest endophyte presence was noted in populations of *F. arundinacea* (98%), *F. rubra* (43%), *F. pratensis* (42%) and *F. ovina* (33%). Also, Lewis (2000) expressed that in many cases in Europe, endophyte incidence in wild populations of *L. perenne, F. arundinacea* and *F. pratensis* was high. Based on the results from many countries, he found that the highest average endophyte incidence was noted in plants of *F. arundinacea* – 95%, while *F. pratensis* and *L. perenne* exhibited about 40% lower (respectively 59% and 49%) infection frequency.

Results of logistic regression (Table 1) proved that the presence of endophytes in given locations was positively related to thermal conditions (variable 6, 7, 9, 10 and 12) and negatively to precipitation (variables 14–6 and 18–21). It has been also claimed by Ju *et al.* (2006) that the main factor influencing the fluctuations in endophyte frequency in plant tissues is temperature. Their study showed that the lower incidence of these fungi in tall fescue was in winter and spring, when average monthly temperatures were often below the minimum temperature for endophyte growth and above the minimum temperature for plant growth.

		Logistic regression results:					
No.	Bioclimatic variable (wording acc. to DIVA-GIS)	chi-	P-value	$R^2$ (%)	regression		
		square	1 vulue	R (70)	10210331011		
1	temperature minimal	5.88	0.015	2.1	+		
2	temperature maximal	1.11	0.293	0.4			
3	annual mean temperature [1]	3.79	0.052	1.4			
4	mean monthly temperature range [2]	5.28	0.022	1.9	_		
5	isothermality (2/7) (* 100) [3]	13.80	0.000	5.0	_		
6	temperature seasonality (STD * 100) [4]	13.77	0.000	5.0	+		
7	max. temperature of warmest month [5]	7.61	0.006	2.7	+		
8	min. temperature of coldest month [6]	0.00	0.953	0.0			
9	temperature annual range (5–6) [7]	5.35	0.021	1.9	+		
10	mean temperature of wettest quarter [8]	10.62	0.001	3.8	+		
11	mean temperature of driest quarter [9]	0.11	0.738	0.0			
12	mean temperature of warmest quarter [10]	11.48	0.001	4.1	+		
13	mean temperature of coldest quarter [11]	0.54	0.464	0.2			
14	annual precipitation [12]	10.99	0.001	4.0			
15	precipitation of wettest month [13]	12.24	0.001	4.4	_		
16	precipitation of driest month [14]	8.42	0.004	3.0	_		
17	precipitation seasonality (CV) [15]	3.28	0.070	1.2	_		
18	precipitation of wettest quarter [16]	12.13	0.001	4.4	_		
19	precipitation of driest quarter [17]	8.37	0.004	3.0	_		
20	precipitation of warmest quarter [18]	12.20	0.001	4.4	_		
21	precipitation of coldest quarter [19]	5.27	0.022	1.9			

Table 1. Results of logistic regression between endophyte presence and bioclimatic variables

It could be predicted, therefore, that global warming will improve the infection and growing conditions for endophytic fungi in more regions of Poland. This effect may be enhanced if warming is associated with less precipitation.

#### References

Gams W., Petroni O. and Schmidt D. (1990) Acremonium uncinatum, a new endophyte in Festuca pratensis. Mycotaxonomy 37, 67–71.

Ju H.J., Hill N.S., Abbott T. and Ingram K.T. (2006) Temperature influences on endophyte growth in tall fescue. *Crop Science* 46, 404–412.

Lewis G.C. (2000) *Neotyphodium* endophytes: incidence, diversity, and host in Europe. *Proceedings of the* 4<sup>th</sup> International Neotyphodium/Grass Interaction Symposium, Soest, Germany, 123–130.

Rudgers J.A. and Swafford A. (2009) Benefits of a fungal endophyte in *Elymus virginicus* decline under drought stress. *Basic and Applied Ecology* 10, 43–51.

Saha D.C., Jackson M.A. and Johnson-Cicalese J.M. (1988) A rapid staining method for detection of endophyte fungi in turf and forage grasses. *The American Phytopathological Society* 2, 78, 237–239.

Siegel M.R., Latch G.C.M. and Johnson M.C. (1985) *Acremonium* fungal endophytes of tall fescue and perennial ryegrass: significance and control. *Plant Diseases* 69, 2, 179–183.

Wäli P., Saikkonen K., Helander M., Lehtimki S. and Lehtonen P. (2000) Seed transmitted endophytic fungi in wild grass populations in Finland. *Proceedings of the 4<sup>th</sup> International Neotyphodium/Grass Interaction Symposium*, Soest-Germany, 93–96.

White J.F.Jr. and Cole G.T. (1985) Endophyte – host associations in forage grasses. I. Distributions of fungal endophytes in some species of *Lolium* and *Festuca*. *Mycologia* 77, 323–327.

Wilson G. (1995) Endophyte – the evolution of a term, and clarification of its use and definition. *Oikos* 73, 247–276.

# Session 4.2.

# Species and habitat diversity of grassland and landscape structure

# Validation of a quick assessment method of agro-ecological structures density on farms

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# Abstract

This paper presents a methodological framework for the quantification of ordinary biodiversity (OB) observed on farms. OB is considered as common species, biotopes and landscapes, because they are neither rare nor threatened species. OB is primarily affected by agronomic factors, such as agricultural practices and landscape factors, such as the organization of agro-ecological structures (AES) on a farm. AES are fixed elements of the landscape, both useful and unproductive, and they provide many ecosystem services. The AES have a role in creating greenveining within agricultural landscapes, but there is yet no simple method to assess the density of AES on a farm. The target of our work is to validate a simple method of assessment of the density of AES by a more accurate method that uses a software georeferencing. Validation is done by comparing two sets of results obtained using the two methods. Both methods were tested in 20 farms, milk and cattle systems in three contrasting regions of France.

Keywords: agro-ecological structures, farm, methodology, greenveining, landscape organization, biodiversity management

# Introduction

There has been a tendency for agriculture to be intensified in recent decades and this leads to a loss of biodiversity. To stop this loss, farmers need information and advice with respect to farming practices: what they should or should not do on their farms. To achieve this goal farmers need tools for assessing biodiversity state on farms. To date, most methods used to assess biodiversity consists of counts, but these methods are cumbersome and expensive, they also require specialists for species identifications. Many authors have shown that the two main factors that affect biodiversity are farming practices and the organizational landscape. A major component of the agricultural landscape organisation is the agro-ecological structures (AES). These structures are fixed element of the landscape that belong to the farm (e.g. hedges, isolated trees, edges etc.). They are important components of the landscape which has an impact on biodiversity. Duelli and Obrist (2003) show that AES are refuges for source arthropod populations in the context of agri-environmental schemes. At a landscape scale, AES are the main driver of bee diversity (Carré et al., 2009). Species richness of vascular plants, birds and arthropods increases with the surface area of AES in European landscapes (Billeter et al., 2008). An important criterion of the agricultural landscape organization is the density of AES on a farm. This information is time-consuming to acquire by GIS methodology (GISM) based on aerial photography. We propose a simplified and quick methodology to sample AES density on each farm investigated. The effectiveness of this method is compared to GIS methodology in order to validate the simplified methodology (SM).

#### Materials and methods

The simplified methodology (SM) needs to apply a grid on the aerial photographs of the farm sampled. The grid consists of 484 squares; each square unit has a surface area of 6 ha. This unit area corresponds to the half-maximum surface area for a plot under cultivation, favourable to the movement of species. Considering the half-maximum surface area, rather than the maximum surface area, keeps a margin to take into account the relief, which is not visible on aerial photography (Bernard *et al.*, 2007).

The number of squares with farm plots with presence of AES (AES on the squares with presence of the plot of the farm; AES on the squares with partial presence of the plot of the farm; AES on the squares adjacent at the squares with presence of the plot of the farm) and the number of squares with farm plots were sampled. A ratio between the two numbers is then calculated.

The GIS method (GISM) aims to obtain a representation of the real world. This representation of the territory of a farm is handled through GIS software (Quantum GIS Development Team, 2009) and associated databases. This representation is obtained by checking aerial photographs. The contours of each farm plots and each AES, like isolated trees, groves, hedgerows and aquatic elements, are then digitalized. A surface is automatically assigned to each drawn component. The extraction of all the surfaces of the farm plots on the one hand, and the different surfaces of AES on the other hand, allows computing the ratio between surfaces of the same kind as a simplified method.

The statistical analysis is performed using the software R 2.13.0 (R Development Core Team, 2010). To analyse the relationship between the two methods, we use Pearson correlation test followed by Spearman rank test.

#### Results

Both methods were tested in twenty farms, with milk and cattle systems in three contrasting regions of France.



Figure 1. A) Relationship between the simple method to assess agro-ecological structures and the GIS method to assess agro-ecological structures, in percentage. Each point represents a farm. B) Mean of each class of agro-ecological structures

There is a significant correlation between the simple method and the GIS method ( $R^2 = 0.35$ , P = 0.006) (Figure 1A). The Spearman rank test is significant (P = 0.008), which means that the highest agro-ecological structures densities obtained by the simple method are the highest agro-ecological structures densities obtained with the GIS method. The results also indicate a predominance of hedgerows among the AES (Figure 1B).

#### Discussion

Both methods are initially very different. One is based on the presence / absence of AES in a grid. The other, much more accurate, is based on the digitalization and calculation of actual surface areas occupied by the AES. However, the results highlighted a relationship between the two methods compared in this paper. These results can be explained by the dominance of hedges, which are visible elements on aerial photographs used in the simplified method for assessment of agro-ecological structures in farm. However, we see in Figure 1A that the values obtained with both methods are not the same order of magnitude. This could explain the low correlation found between the two methods. The values obtained by the GIS method vary from 0 to 20 percent. The agro-ecological structures density obtained with the simple method cannot be used as such. The simplified method could be a way to access more easily and quickly information which can be obtained more precisely by the GIS method.

On the other hand, all farms in the sample have a density of agro-ecological structures greater than 30% for data of the simplified method. This can be explained by the fact that they are all milk and cattle systems. Indeed, we assume that agro-ecological structures are mainly located on or near permanent grasslands, which are more frequent in livestock systems. It would be interesting to test these methodologies on crop systems.

#### Conclusions

The GIS method is not very complicated to implement, but the digitization of farm plots and AES is time consuming and requires precision and rigor. So, the validation of the simplified method allows an overall and quick assessment of AES on the farm. This simplified method has the advantage of being achievable by anyone without training, like end-users or farmers.

#### References

Bernard J.L., et al. (2007) Productions végétales, pratiques agricoles et faune sauvage: pour une agriculture performante et durable. Union des industries de la protection des plantes, 251 pp.

Billeter R., Liira J., Bailey D. et al., (2008) Indicators for biodiversity in agricultural landscapes: a pan-European study. *Journal of Applied Ecology* 45, 141–150.

Carré G., Roche P., Chifflet R. et al. (2009) Landscape context and habitat type as drivers of bee diversity in European annual crops. Agriculture, Ecosystems and Environment 133, 40–47.

Duelli P. and Obrist M.K. (2003) Regional biodiversity in an agricultural landscape: the contribution of seminatural habitat islands. *Basic and Applied Ecology* 4, 129–138.

Quantum GIS Development Team (2009) *Quantum GIS Geographic Information System*. Open Source Geospatial Foundation Project. http://qgis.osgeo.org.

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

# Evaluation of species diversity of pasture sward under different grassland management

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# Abstract

The influence of different grazing regimes and fertilisation on species diversity was investigated in a long-term study during the years 2003 to 2010 in the locality of Rapotin (Czech Republic). The treatments were arranged in a factorial design in which absence or presence of fertilisation ( $N_{100}$ PK, pure nutrients) and different number of grazing periods (two, three, and four per year) were imposed. A highly significant effect of fertilisation was found, in which non-fertilized treatments showed a higher mean value of diversity index (DI = 5.72) than fertilized treatments (DI = 4.60). The moderate treatment (three grazing periods per year) without mineral fertilisation showed the highest value of the diversity index in the mean of years (DI = 6.08), which indicates a well-balanced community and better ecological conditions.

Keywords: mineral fertilisation, pasture experiment, permanent grassland, species diversity

#### Introduction

In the Czech Republic, grazing is typical of less-favoured areas where grasslands were established a long time ago. In these areas, replacement of ruminant farming by other activities is impossible in practice (Harvieu, 2002). Grazing by suckler cows can be a good option for grassland management in harmony with productive and non-productive functions, because this cattle category has relatively low nutritional requirements. According to the latest official statistics, suckler cow numbers in the Czech Republic rose by 60% to 168 000 head between 2002 and 2010; this accounts for 30.4% of the total cow production (552 000 head) (Kvapilík *et al.*, 2010). However, many organisational, scientific and economic issues need to be addressed to foster further development in the field of low input and organic grazing. This study aimed at exploring the species diversity as a response to the type of grassland management (grazing utilisation and mineral fertilisation) in the Czech Republic.

#### Materials and methods

For the experimental evaluation, we used a data set of six plots established in 2003 on permanent grassland sites in the locality of Rapotin, representing grasslands with different intensity of utilisation and different fertilisation rates. The locality is situated at 340 m a.s.l. The grassland vegetation is related to the alliance *Arrhenatherion*. The plots, with an area of 200 m<sup>2</sup> (visually divided into 4 replicates of 50 m<sup>2</sup>) were grazed rotationally (paddock grazing system) by heifers from the suckler-cow breeding system with three different intensities of grazing utilisation (four – P4, three – P3, or two – P2, grazing periods per year). Each type of utilisation was established in a treatment with or without additional application of mineral fertilizers (N<sub>100</sub>P<sub>30</sub>+K<sub>60</sub>, pure nutrients). Phosphorus was applied as superphosphate, and potassium as potassium salt, at one rate in the spring. Ammonium salt with limestone was used as a nitrogen fertilizer, whereas N fertilization strategy was a split-application with 50% of N in spring and 50% of N in summer. The treatments were arranged in a factorial design in which absence or presence of fertilisation (NPK) and different number of grazing periods were imposed, i.e. a  $2\times3$  factorial with paddocks as the experimental units (4 per treatment combination). In practice, the experimental plots were part of a larger pasture (total area with open plot: approximately 1 ha), on which 5 heads of heifers (350-400 kg live weight) were allowed to graze continually during each vegetation season and they were supplemented only with mineral salt. In certain dates of grazing periods the experimental plots. After each grazing period residual forage was mown and removed from the experimental plots. Afterwards, the sward was left to re-grow for the next grazing. The botanical composition of the treatments was estimated each year before the first grazing period by means of the projective dominance method. Species diversity in a community was measured by Simpson's diversity index (DI) (Begon *et al.*, 1997) modified according to Klimeš (2000):

$$DI = \frac{1}{\sum_{i=1}^{s} p_i^2}$$

where  $p_i$  is projective dominance of the *i*th species and S is total number of species (richness). The overall data obtained during the observation years were processed using software STA-TISTICA CZ v. 10. The differences between mean results of the species diversity indexes were statistically evaluated using the method of three-way analysis of variance, whereas the factors intensity of utilisation (number of grazing periods), fertilisation and year were included. The significance of the differences between treatments was assessed by the Tukey's HSD test at a significance level of 0.05.

#### **Results and discussion**

A highly significant effect of fertilisation was observed, with unfertilized treatments showing a higher mean value of diversity index (DI = 5.72) than fertilized (DI = 4.60) (Tables 1 and 2). Similarly, Hrevušová *et al.* (2009) found low values of species diversity in grasslands fertilized with  $N_{400}$ PK, even after 16 years since the last application. A significant effect of years was also found, which could be caused by variable weather conditions during our monitoring (data not shown in this paper). The effect of intensity of utilisation (number of grazing periods) was also significant, but had a lower impact with respect to fertilisation or year. The Simpson's diversity index decreased with decreasing number of grazing periods per year – from 5.31 to 4.90. Overall, the highest value of DI, 6.08, was achieved in the moderate treatment (three grazing periods per year) without mineral fertilisation. It indicates a well-balanced community and better ecological conditions.

Effect	Degrees of freedom	F-ratio	<i>P</i> -value	
Grazing periods per year	2	3.66	0.02813	
Fertilisation	1	71.69	< 0.0001	
Year	7	29.38	< 0.0001	
Year $\times$ grazing periods per year	14	15.36	< 0.0001	
Year $\times$ fertilisation	7	3.71	0.00102	
Grazing periods per year × fertilisation	2	7.96	0.00053	
Year $\times$ grazing periods per year $\times$ fertilisation	14	3.36	< 0.0001	

Table 1. Results of ANOVA analyses of Simpson's diversity indexes (n = 192)

Treatment	Year						Maan	SEM		
А	2003	2004	2005	2006	2007	2008	2009	2010	Mean	SEIVI
four	3.13 <sup>a</sup>	3.83ª	3.97ª	3.18 <sup>a</sup>	5.66 <sup>a</sup>	6.52ª	7.22ª	8.95ª	5.31ª	0.20
three	3.96ª	5.41 <sup>b</sup>	4.79ª	4.11 <sup>ab</sup>	4.68 <sup>b</sup>	6.84ª	5.37 <sup>b</sup>	6.90 <sup>b</sup>	5.26 <sup>ab</sup>	0.82
two	5.70 <sup>b</sup>	4.83 <sup>b</sup>	4.72 <sup>a</sup>	4.67 <sup>b</sup>	5.34 <sup>ab</sup>	5.80 <sup>a</sup>	3.51°	4.66 <sup>c</sup>	4.90 <sup>b</sup>	0.66
В										
non-fertilisation	5.02ª	4.94 <sup>a</sup>	4.64 <sup>a</sup>	4.35 <sup>a</sup>	5.55ª	7.27ª	5.93ª	8.04 <sup>a</sup>	5.72ª	0.26
$N_{100}P_{30}K_{60}$	3.51 <sup>b</sup>	4.45 <sup>a</sup>	4.34 <sup>a</sup>	3.62 <sup>b</sup>	4.91 <sup>b</sup>	5.5 <sup>b</sup>	4.81 <sup>b</sup>	5.64 <sup>b</sup>	4.60 <sup>b</sup>	0.37

Table 2. Diversity of grass stands depending on number of grazing periods per year (part A) and fertilisation (part B)

Means followed by equal letters in columns do not differ by Tukey's HSD test (P > 0.05).

Our results are in accordance with the intermediate disturbance hypothesis (IDH) which predicts that species richness and species diversity, given a suitable range of environmental disturbance levels, should peak at intermediate levels (Rejmánek *et al.*, 2004). Both extremes – intensive way of grassland management as well as grassland abandonment – could have a negative impact from the viewpoint of species diversity, as was documented also by Zarzycki and Mistral (2010). Further, we can state that nitrogen fertilization of pastures cannot always be recommended as a suitable step of grassland management, particularly from the viewpoint of diversity maintenance. Pasture fertilization should be more controlled by careful consideration of individual pasture goals (production for animals, time of forage needs, species present, expected methods of management). If there is a goal to maintain species diversity, then grasses must be kept slightly nitrogen deficient.

#### Conclusions

On the basis of our results we can state that under the extreme level of basic ecological factors (nutrition regime) and under the extreme level of grazing utilisation (intensive or extensive), species diversity of the grassland community is reduced. Maintenance of pastures and meadows in their species richness and diversity requires appropriate management that is adequate for site conditions and the status of the sward. Species diversity and ecosystem stability maintenance can be ensured only by systematic grassland management.

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# References

Begon M., Harper J.L. and Towsend C.R. (1997) *Ecology-individuals, populations and communities*. Palacky Univ., Olomouc, 949 pp.

Harvieu B. (2002) Multi-functionality: a conceptual framework for a new organisation of research and development on grasslands and livestock systems. *Grassland Science in Europe* 7, 1–2.

Hrevušová Z., Hejcman M., Pavlů V., Hakl J., Klaudisová M. and Mrkvička J. (2009) Long-term dynamics of biomass production, soil chemical properties and plant species composition of alluvial grassland after the cessation of fertilizer application in the Czech Republic. *Agriculture, Ecosystems and Environment* 130, 123–130.

Klimeš F. (2000) Dynamics of species richness of floodplain meadowws. *Rostlinná Výroba* 46(5), 198–208. Kvapilík J., Růžička Z. and Bucek P. (2010) Ročenka 2009 – Chov skotu v České republice (Cattle Breeding in the Czech Republic). ČMSCH, SCHČSS, SCHHS, ČSCHMS, Prague, 96 pp.

Rejmánek M., Rejmánková E. and Holzner W. (2004) Species diversity of plant communities on calcareous screes: the role of intermediate disturbance. *Preslia* 76, 207–222.

Zarzycki J. and Mistral A. (2010) Abandonment of farming practices: impact on vegetation. *Grassland Science in Europe* 15, 133–135.

# Habitat preferences of plant associations from *Molinio-Arrhenatheretea* class described in the 'The Bug Ravine Landscape Park' by GGE biplot analysis

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# Abstract

Six associations from the *Molinio-Arrhenatheretea* class occurring in 'The Bug Ravine Landscape Park' were described using the Braun-Blanquet method. The collected data were analysed based on the GGE biplot method with the use of the Ellenberg indicator values. It allowed to specify the habitat preferences of the described communities.

Keywords: grassland communities, ecological scale, vegetation surveys

# Introduction

Semi-natural plant communities from *Molinio-Arrhenatheretea* R. Tx. 1937 class are widespread in Europe (Ellenberg and Strutt, 2009). This syntaxon consists of a large number of associations which are connected with different site conditions and formed under different types of grassland management. There are still many difficulties in defining the habitat conditions in which those communities form and exist.

In the presented study, 6 associations from *Molinio-Arrhenatheretea* class were analysed. Their classification (Nowiński, 1967; Matuszkiewicz, 2008) is as follows:

Order: Molinietalia caerulae W. Koch 1926

Alliance: Calthion palustris R. Tx. 1936 em. Oberd. 1957

Associations: Deschampsietum caespitosae Horvatić 1930;

Holcetum lanati Issler 1936

Alliance: Alopecurion pratensis Pass. 1964

Association: Alopecuretum pratensis (Regel 1925) Steffen 1931

Order: Arrhenatheretalia Pawł. 1928

Alliance: Arrhenatherion elatioris (Br.-Bl. 1925) Koch 1926

Association: Arrhenatheretum elatioris Br.-Bl. ex Scherr. 1925

Poo -Festucetum rubrae Fijałk. 1962

Alliance: Cynosurion R. Tx. 1947

Association: Lolio-Cynosuretum cristati R. Tx. 1937.

The aims of the study were: a) to describe the habitat preferences of the chosen plant associations that belong to *Molinio-Arrhenatheretea* class and are present in the area of 'The Bug Ravine Landscape Park'; and b) to determine the usefulness of the GGE (Genotype and Genotype-by-Environment interaction effects, Yan and Kang, 2003) biplot method for analysing the habitat preferences of plant communities.

# Materials and methods

Six associations from the *Molinio-Arrhenatheretea* class occurring in 'The Bug Ravine Landscape Park' were described using a total of 241 relevés collected with the use of the Braun-Blanquet method (Braun-Blanquet, 1964). They were: *Arrhenatheretum elatioris* (16 relevés), *Lolio-Cynosuretum* (42), *Poo-Festucetum* (45), *Holcetum lanati* (30), *Deschampsietum caespitosae* (58) and *Alopecuretum pratensis* (50). During classification process, the
Jaccard distance and Ward's clustering were used. The nomenclature of plant species were named according to Mirek *et al.* (2002) while the nomenclature of plant communities follows Matuszkiewicz (2008) and Nowiński (1967). For each of the relevé Ellenberg indicators (L – light, F – moisture, R – soil pH, N – soil nitrogen, T – temperature, K – continentality) were calculated. These parameters were described by GGE biplot method which is often used for analysing the relations between yields of genotypes growing in different agro-environmental conditions. The computations were performed in the R environment (2008). The results were visualized on a GGE biplot (Gabriel, 1971).

#### **Results and discussion**

In brief, the ecological characteristics of the described associations based on the generated biplot (Figure 1) are as follows:



Figure 1. GGE biplot of the first and the second principal component (PC1 and PC2, respectively) based on phytosociological relevés described using Ellenberg indicator values (are denoted by adequate letters) calculated for each relevé. The biplot clarify 51% of the variability (30% and 20% for axes respectively). The ellipses have been plotted in order to make the plot clearer. Each of the ellipses contains 80% point of respective associations and has minimal area

*Deschampsietum caespitosae* – it does not appear in the fertile, well-lit habitats. Apart from that it has a wide ecological range. Hence, a large number of subassociations of that syntaxon are noted (Rodwell, 1998; Botta-Dukát, 2005). The habitat preferences of this community to a large extent coincide with the preferences of *Holcetum lanati*. Some researchers (Rodwell, 1998) even separate *Holcus lanatus* – *Deschampsia caespitosa* community. According to data, the *Holcetum* more often occurs in better lit, warmer places while the *Deschampsietum* shows higher level of tolerance to soil moisture.

*Holcetum lanati* – is characterized by the highest dispersion of occurrence which reflects the ambiguous classification of this (Nowiński, 1967).

*Alopecuretum pratensis* – usually occurs in damp, nutrient-rich places (Matuszkiewicz, 2008) as it could be noted in the Bug valley. Unfavourable transformations of this community are often connected with lack of river flooding and results in appearance of xerothermic species (Kryszak and Grynia, 2001).

*Arrhenatheretum elatioris* – the requirements of this association seems to be well-defined: neutral to alkaline, nutrient rich soil, well-lit, dry or of moderately wet places, though a much greater variability of *Arrhenatheretum* occurence is known (Ellenberg and Strutt, 2009). *Poo-Festucetum rubrae* – by some authors is regarded as degraded form of *Arrhenatheretum elatioris* dominating by *Poa pratensis* and *Festuca rubra* and occurred on non-fertilized, drier sites (Matuszkiewicz, 2008). The obtained results do not contradict that hypothesis. *Lolio-Cynosuretum* – it represents communities of mesic pastures. Its high light requirements are connected with the large share of low, grazing-tolerant plants. Figure 1 shows that some patches of *Lolio-Cynosuretum* were found on nutrient-poor, acidic soils, while another preferred nutrient-rich, alkaline ones. Such a different habitat requirements within the community seem to be associated with a broad ecological scale of this syntaxon (Trąba *et al.*, 2008).

## Conclusions

The use of GGE biplot analysis allowed describing habitat preferences of plant communities in a clear and comprehensive way. All the analysed associations show clearly different preferences of combination of ecological traits of habitats. They fit into two groups in terms of site humidity and exposure to sunlight: 1) associations which prefer semi-shade, damp habitats: *Alopecuretum pratensis* and *Deschampsietum caespitosae* (both placed in *Molinion* alliance); 2) associations which prefer moderately moist, well-lit places: *Arrhenatheretum elatioris*, *Lolio-Cynosuretum* and *Poo-Festucetum* (grouped in *Arrhenatheretalia* order); and in terms of soil fertility level and soil pH they were grouped as follows: 1) association of nutrient-rich, neutral to alkaline habitats: *Arrhenatheretum elatioris*, *Alopecuretum prtensis*; 2) associations of nutrient-poor, acidic habitats: *Holcetum lanati*; 3) associations with a wide adaptation to habitat conditions – *Poo-Festucetum*, *Lolio-Cynosuretum* and *Deschampsietum caespitosae*.

# References

Botta-Dukát Z., Chytrý M., Hájková P. and Havlová M. (2005) Vegetation of lowland wet meadows along a climatic continentality gradient in Central Europe. Preslia, Praha, 77, 89–111.

Braun-Blanquet J. (1964) *Pflanzensoziologie. Grundzüge der Vegetationskunde.* Sprinter-Verlag, Wien-New York.

Gabriel K.R. (1971) The biplot graphic display of matrices with application to principal component analysis. *Biometrica* 58, 453–467.

Ellenberg H. and Strutt G.K. (2009) *Vegetation ecology of Central Europe*. Cambridge University Press, pp. 756. Kryszak A. and Grynia M. (2001) Floristic diversity and economic value of the *Alopecuretum pratensis* association in Western Poland. *Grassland Science in Europe* 6, 164–166.

Matuszkiewicz W. (2008) Przewodnik do oznaczania zbiorowisk roślinnych Polski. Wyd. Nauk. PWN in Polish. Mirek Z., Piękoś-Mirkowa H., Zając A., Zając M. et al. (2002) Flowering plants and pteridophytes of Poland. A checklist. Instytut Botaniki im. W. Szafera, PAN, Kraków, pp. 340, in Polish.

Nowiński M. (1967) *Polskie zbiorowiska trawiaste i turzycowe*. PWRiL, Warszawa, p. 107–108 in Polish. R Development Core Team. (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rodwell J.S. (1998) British Plant Communities: Grasslands and montane communities, Cambridge University Press, pp. 540.

Trąba Cz., Wolański P. and Oklejewicz K. (2008) Floristic diversity and sward use value of *Lolio-Cynosuretum* association in the San river valley. *Annales UMCS* 63(2), sectio E, 67–73.

Yan W. and Kang M.S. (2003) *GGE Biplot Analysis: A Graphical Tool for Breeders, Geneticists, and Agronomists.* Boca Raton, FL, CRC Press, pp. 288.

# Controlling broom (*Cytisus scoparius*) encroachment by browsing based on shrub demographic strategy

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# Abstract

Scotch broom (*Cytisus scoparius*) is a very dominant species, often having undesirable environmental impacts. Browsing appears to be a powerful tool to control it, since it is targeted at the juvenile stage, which is the most sensitive in the demographic strategy of this species. A seven-year experiment on a natural Scotch broom shrubland in a French Pyrenean rangeland assessed the effects of three different browsing intensities (two levels of manual stem biomass removal simulating light and heavy browsing *vs* an untreated control) on juvenile survival, morphology and reproductive success at a young adult stage. These browsing strategies, representing pertinent management practices, were repeated over a five-year period. We also analysed the recovery of broom plants, i.e. surviving young adults after two years without browsing. Our results demonstrate that browsing the young plants is a promising strategy for controlling broom.

Keywords: juvenile, Scotch broom, shrub control, shrubland, target stage

# Introduction

Scotch broom (*Cytisus scoparius*) is a long-lived shrub that can reach very high densities, representing up to 90% of the biomass of the plant community (Peterson and Prasad, 1998). Browsing by domestic herbivores has been proposed to control its spread, despite the lack of knowledge on how this might work. A previous study (Magda *et al.*, 2009), modelling its demographic strategy, revealed that the survival of juveniles (plants aged >1 year from germination until reproductive stage, i.e. 3–4 years) is the key parameter for the population growth rate. The next step after identifying this potentially sensitive growth stage is to understand how browsing can affect target life stages in regulating population demography. Hence the aim of this study was to describe the effects of different simulated browsing intensities repeated over five years. It focused initially on juveniles and on the demographic and morphological characteristics of Scotch broom. Further, in order to develop an effective plan for controlling its dominance over time, we studied the effects of browsing intensities on the subsequent potential for shrub recovery after a period without browsing.

# Materials and methods

The experiment was conducted over seven years, from 2004 to 2010, on a private property in the southwest Ariège-Pyrénées region, France, at a mean altitude of 1300 m. The study area was a natural Scotch broom shrubland. A 0.5 ha plot was established within a large area of broom, and fenced to keep out stray herbivores. 330 juvenile Scotch broom plants (aged 1–2 years) were randomly selected from the entire population in 2004. Three browsing intensities were applied to these using clippers developed to simulate the bite of a sheep's

jaw. The bite simulations, described in Agreil and Meuret (2004), were used to define the length and number of leafy branches sectioned at each bite. The plants were either left untouched (T0, control; n = 126) or had 50% (T50) or 90% (T90) of their total edible stem biomass removed at two cuttings per year for "light-intensity browsing" (n = 127) and "high-intensity browsing" (n = 77) treatments, respectively. The three different browsing intensities were repeated for 5 years, from 2004 to 2008. We compared survival rates, morphology (height and collar diameter) and reproductive success (fecundity) of broom plants during and after the browsing period (2009 and 2010). Non-parametric statistics, i.e. Chi square ( $\lambda^2$ ) and Kruskal-Wallis test, were used (Statgraphics, v5.0, Manugistics Inc., USA) to test for between-treatment variability in annual survival rates and in reproductive output, respectively.

## **Results and discussion**

Figure 1 shows the year-by-year development in survival rates of juvenile and young adult plants (from 2005) for each treatment. Survival rates for T0 ranged from 0.97 in 2004 to 0.77 in 2010. For T50, survival rates ranged from 0.92 to 0.29. For T90, survival rates dropped drastically, from 0.93 in 2004 to 0.05 in 2010. Between-treatment variability only became significant in 2007 (Table 1). Our study demonstrates a threshold for the survival of young broom plants, which in practical terms means that they have to be grazed for at least three years before becoming noticeably reduced. This also indicates that juveniles had high survival rates, and generally reached the young adult stage in all treatments. However, under high-intensity browsing, less than 10% of broom individuals reached complete reproductive maturity (>7 years). Further, compared to the control treatment, browsing effects on broom survival rate remained effective during the first two years after the cessation of repeated browsing (from 2008 onwards).



Figure 1. Survival rate and percentage of reduction compared to non-cut plants of height (H) and collar diameter (solid lines) per treatment (T0, non-cut during the year; T50, 50% of broom biomass cut during the year; T90, 90% of broom biomass cut during the year), and per year. Survival rates were calculated as the number of surviving individuals/number of total individuals. The dashed line shows the last year of browsing treatments

When analysing height and collar diameter of broom plants, we found significant differences (P < 0.05) among treatments. For instance, in 2008 broom plants had a mean height of 130±4.22, 64±4.22 and 48±5.19 cm, and a mean collar diameter of 27±7.50, 12±3.82 and 13±5.40 mm for T0, T50 and T90, respectively. After 5 years of experimentation, repeated browsing led to a >50% reduction in plant size (Figure 1). This suggests that because they probably contain more palatable twigs and plant organs below the maximum browsing height (e.g. 120 cm for ewes), broom plants are continuously targeted for browsing. However, in the early years after browsing cessation, differences between surviving cut (T50 and T90) and non-cut plants seemed to decrease (on average, a 38% reduction in 2010, Figure 1).

Year	$\lambda^2$	Р
2004	2.52	ns (0.283)
2005	1.71	ns (0.425)
2006	4.52	ns (0.104)
2007	15.79	***
2008	101.0	***
2009	106.7	***
2010	110.2	***

Table 1. Chi-squared ( $\lambda^2$ ) test for between-treatment survival rate variability (df = 2) over seven consecutive years (2004–2010)

Between 2005 and 2009, the average number  $(\pm se)$  of seeds per adult plant was  $376\pm 220.0$ ,  $16.2\pm13.29$  and  $0.15\pm0.075$  for T0, T50 and T90 (P = 0.022), respectively. Thus, compared to the control treatment (T0), light (T50) and heavy (T90) browsing led to a 96% and nearly 100% reduction in reproductive output, respectively. The differences between treatments T50 and T90 were not significant (P = 0.205). We have presented average all-vear data since, unlike survival rates, there was a rapid drop in the reproductive output of young adult plants after treatment initiation. The drastic reduction in reproductive output under light or heavy-intensity browsing was achieved by the browsing of new shoots elongated from late summer to autumn, which are responsible for seed production the following year. This reduction was probably enhanced by changes in priority resource allocation for the replacement of vegetative shoots (photosynthetic material), and a subsequent reproductive delay in surviving plants. The net result would be a smaller seed production over the plant's lifetime. In 2010, however, the light browsing treatment led to a mere 76% reduction in reproductive output compared to the control (n = 35). Thus, seed production can reappear even after long periods of browsing. Since the number of survivors for T90 was very low in 2010 (n = 4), we have not considered reproductive output for this treatment.

# Conclusions

This multi-year survey demonstrates that focusing browsing on twigs of young plants can be an efficient strategy for controlling broom, since repeated browsing significantly affected the survival rates of juveniles and their subsequent young adults. This regulation of the recruitment process is also enhanced by limiting reproduction and the provision of the seed bank. While the light- and heavy-intensity browsing levels used in this study both appear to be very high, we argue that the intensity of consumption applied here is a reliable management technique, since twigs continue to be easy targets due to their small size and (probably) greater palatability. Additionally, the consumption rates tested in this study are likely more applicable to broom stands with lower density and a higher proportion of juveniles. However, especially after a long period of light browsing, broom plants seem to show a potential for recovery. Nevertheless, a better assessment of the stability of the effects of previous browsing requires both a longer study period, and analysing the impact of these effects on the population growth rate.

#### References

Agreil C. and Meuret M. (2004) An improved method for quantifying intake rate and ingestive behaviour of ruminants in diverse and variable habitats using direct observation. *Small Ruminant Research* 54, 99–113. Magda D., Chambon-Dubreuil E., Agreil C., Gleizes B. and Jarry M. (2009) Demographic analysis of a dominant shrub (*Cytisus scoparius*): Prospects for encroachment control. *Basic and Applied Ecology* 10, 631–639.

Peterson D.J. and Prasad R. (1998) The biology of Canadian weeds (*Cytisus scoparius* (L.) Link). *Canadian Journal of Plant Science* 78, 497–504.

# Polish Carpathian grasslands vegetation survey

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# Abstract

Semi-natural grasslands in the Carpathians are among the most diverse plant communities in Poland. However, changes in their management and abandonment of farming practices have caused rapid changes in their botanical composition. The grassland vegetation data from the past are no longer up to date. Therefore, the survey of the present meadow vegetation in the Polish Carpathians was started in 2009. A regional database of present and archival phytosociological relevés is being created. Based on the data collected so far, three main groups of plant communities can be distinguished. In submontane areas, grasslands are represented mainly by false oat-grass meadows (*Agrostietum*) are to be found only at higher altitudes in some parts of the Polish Carpathians. Poor mat-grass swards (*Nardetum*), widespread in the past, have almost disappeared; the main reason being the intensity of agriculture. Cattlerearing is usually based on fodder obtained from grassland created on former arable land. Such grasslands consist mainly of cultivated grasses and are species-poor false oat-grass meadows.

Keyworlds: mountain grasslands, vegetation databases, phytosociology

# Introduction

Plant communities have been shaped by human land-use management, and thus they are extremely responsive to the changes in the forms and intensity of use. The changes in agricultural management occurring in Poland in recent decades have resulted in the abandoning of many permanent grasslands and creating new ones on former arable lands. Some plant communities have disappeared, and new ones emerged. The existing data concerning meadows vegetation in the Carpathians is often out of date. The coverage by relevés made in meadows is extremely uneven: the areas of great values in terms of nature (e.g. protected areas) are over-represented, whereas for others there are almost no data available. The knowledge of the current state of vegetation is, however, essential to making correct environmental management decisions, as well as a valuable resource for scientific research. The objective of this project is to create a supra-regional database for meadows and pastures in the Polish Carpathians. Collecting relevés for such databases is done in many countries, particularly in Europe (Dengler *et al.*, 2011). The data gathered in these databases makes possible to describe (classify) communities at local and regional levels, assess the rate and direction of changes, and can be used in evaluation of natural areas (Schaminee *et al.*, 2009; Rozbrojová *et al.*, 2011).

# Materials and methods

The Polish Carpathians form a 330 km long arch along the southern border of Poland, covering an area of 19.6 thousand km<sup>2</sup>. The range includes sub-montane areas as well as high-mountain ranges. Grasslands occur within the range of elevations from 250 m a.s.l. (in inner mountain valleys) to 1900 m a.s.l. (Warszyńska, 1995). The density of human population and the level of economic development vary immensely between particular regions.

In this project, apart from inventorying all available relevés made after 1960 under the Turboveg programme (ca. 2400 relevés made in the Polish Carpathians), data is being collected by the routine methods of plant sociology, from the areas not yet sufficiently investigated to date. Until the present time, 700 out of 1200 planned relevés have been completed. The preliminary analysis presented in this paper covers also the relevés made after the year 2000, and a total of 1178 relevés were analysed.

#### **Results and discussion**

Meadows and pastures in the Polish Carpathians show remarkable diversity in terms of species composition and reflect the historical and economic land-use pattern connected with habitat-related factors. The data collected until the present have provided a basis to distinguish three principal types of communities.

The most common in the sub-montane and low mountain situations (usually below 600 m a.s.l.) are the communities of fertile cultivated meadows, closely related to lowland false oatgrass meadow association (*Arrhenatheretum elatioris*). Among the relevés under consideration, as many as 49% were allocated into this group. The dominant species are usually sown grasses, orchard grass (*Dactylis glomerata*), meadow foxtail grass (*Alopecurus pratensis*), and false oat grass (*Arrhenatherum elatius*). There is also a major proportion of red clover (*Trifolium pratense*). The meadows of this type are moderately rich in species (average of 32.3 species per relevé) (Table 1).

Community	Oat-grass	meadow	Bent-gra	ss meadow	Mat-grass swards		
No. of relevés	573		463		142		
	Constance*	Phi coef-	Constance	Phi coef-	Constance	Phi coef-	
	Constancy*	ficient**	Constancy	ficient	Constancy	ficient	
Diagnostic species							
Arrhenatherum elatius	III	48.2	Ι	_	Ι	_	
Ranunculus repens	IV	46.9	II	_	Ι	_	
Taraxacum officinale	IV	44.3	II	1	Ι	_	
Crepis biennis	III	45.9	Ι	_	Ι	_	
Alopecurus pratensis	III	42.1	Ι	_	Ι	_	
Heracleum sphondylium	III	41.7	II	-	Ι	-	
Poa trivialis	III	40.6	Ι	_	Ι	_	
Holcus lanatus	III	38.6	II	_	Ι	_	
Dactylis glomerata	V	42.5	III	11.3	Ι	_	
Trifolium pratense	IV	41.8	III	7.8	Ι	_	
Festuca pratensis	IV	35.7	III	10.3	Ι	_	
Agrostis <sup>°</sup> capillaris	III	-	V	27.4	V	15.4	
Leontodon hispidus	III	8.5	III	25.3	I	-	
Gladiolus imbricatus	Ι	_	Ι	24.9	I	-	
Nardus stricta	Ι	_	II	_	V	65.7	
Potentilla erecta	Ι	_	III	5.1	V	51.6	
Carex pilulifera	Ι	_	Ι	_	III	47.7	
Veronica officinalis	Ι	_	II	_	III	37.1	
Hieracium vulgatum	Ι	_	Ι	_	II	34.9	
Constant species							
Anthoxanthum odoratum	IV	9.5	IV	5.9	IV	-	
Rumex acetosa	V	16	IV	8.6	III	-	
Achillea millefolium	IV	12.2	V	15	III	-	
Plantago lanceolata	IV	20.4	IV	17.8	III	_	
Trifolium repens	IV	23.5	IV	19.4	II	-	
Ranunculus acris	IV	19.1	IV	19.5	II		
Festuca rubra	III	-	V	16.3	V	13.4	
Hypericum maculatum	III	-	IV	12.2	IV	9.2	
	``````````````````````````````````````	T 1 0004	TT 00 4	0.0/ 111 4.0	(00/ III	60 000/ N	

#### Table 1. Diagnostic and constant species for three main types of meadows

\*Constancy (frequency of occurrences): I - 1-20%, II - 20-40%, III - 40-60%, IV - 60-80%, V - 80-100%.

\*\*Phi coefficient is the positive fidelity value between a particular vegetation unit and a species (Tichy, 2002).

They have developed on fertile soils of moderate moisture content, usually on former arable land and in river valleys. The second type of community is the bent-grass meadows (closely related to *Gladiolo-Agrostietum*) which are widespread mainly in the 400–900 m a.s.l. altitudinal zone. Apart from species occurring practically in all meadow communities of the Carpathians, such as red fescue (*Festuca rubra*), common dock (*Rumex acetosa*) and sweet vernal grass (*Anthoxanthum odoratum*), the species characteristic of meadows include: common bent grass (*Agrostis capillaris*), hawkbit (*Leontodon hispidus*), and meadow gladiolus (*Gladiolus imbricatus*) (Table 1). Very often the physiognomy of the community is determined by various species of lady's mantle (*Alchemilla* sp. div.). The number of species per relevé (34.9 on average) is usually higher then in oat-grass meadow, and protected and rare species and numerous species of the orchid family occur very often.

The third type of community, the mat-grass swards, survived in the Carpathians only in 12% of all relevés, and they were obtained mainly from the lower and upper mountain zones. The occurrence of mat-grass swards depends on certain conditions: poor, acid soil and the effects of long time under pastoral management. Mat grass (*Nardus stricta*) is the species predominating in the sward. It is usually accompanied by other species typical of poor habitats, such as: tormentil (*Potentilla erecta*), pill-headed sedge (*Carex pilulifera*) and wood speedwell (*Veronica officinalis*). Most of these areas have been taken out of use and usually overgrown by bilberry (*Vaccinium myrtillus*). The average number of species per relevé is significantly lower than that in oat-grass meadows and bent-grass meadows (Table 1).

#### Conclusions

The studies completed to date show that the oat-grass meadows presently predominate in the Polish Carpathians. There is a great diversity in species composition within the bent-grass meadows and rather slight differences between them and oat-grass meadows. The alterations in land use are probably the main reason for this observation. Fodders are now obtained mainly from meadows and pastures introduced on former arable lands where species-poor associations with predominance of cultivated grass species have been established. Most of permanent grassland has been either artificially afforested or succumbed to secondary forest succession. This process regards mostly the mat-grass swards but also species-diverse bent-grass associations, which survived only in some parts of the Carpathians.

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#### References

Dengler J., Jansen F., Glöckler F., Peet R.K., DeCáceres M., Chytrý M., Ewald J., Oldeland J., Lopez-Gonzalez G., Finckh M., Mucina L., Rodwell J.S., Schaminée J.H.J. and Spencer N. (2011) The Global Index of Vegetation-Plot Databases (GIVD): a new resource for vegetation science. *Journal of Vegetation Science* 22, 582–597.

Rozbrojová Z., Hájek M. and Hájek O. (2010) Vegetation diversity of mesic meadows and pastures in the West Carpathians. *Preslia* 82, 307–332.

Schaminee J.H.J., Hennekens S.M., Chytry M. and Rodwell J. (2009) Vegetation-plot data and databases in Europe: an overview. *Preslia* 81, 173–185.

Tichý L. (2002) JUICE, software for vegetation classification. *Journal of Vegetation Science* 13, 451–453. Warszyńska J. (ed.) (1995) *Karpaty Polskie*. Uniwersytet Jagielloński, Kraków.

# Factors influencing butterfly diversity in a long-term experiment differing in grazing intensity

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# Abstract

Reducing grazing intensity offers opportunities for maintaining and enhancing biodiversity. By creating a heterogeneous mosaic of patches differing in sward height and vegetation composition, extensive grazing provides good opportunities for diverse butterfly assemblages. Here, we report long-term effects of three grazing intensities (adjusted by target compressed sward heights: 6 cm and 12 cm since 2002 and additionally 18 cm since 2005) arranged in a randomized block design with three replicates, on a continuously stocked pasture grazed by cattle in the Solling Uplands, Germany. Data on butterfly species richness and abundance were collected from 2003 and 2004 and again in 2010 and 2011 on three 50 m-transects per paddock on three occasions in summer. Results suggest a lenient grazing intensity to be most advantageous for butterflies. The findings are presented in relation to abundance of flower heads as well as to sward surface height and its heterogeneity.

Keywords: butterflies, Lepidoptera, management, species richness, abundance

# Introduction

Butterflies (Lepidoptera) are declining across grasslands in Europe (Van Swaay *et al.*, 2006). One of the key management practices aiming at insect and, in particular, butterfly diversity is to reduce grazing intensity (Kruess and Tscharntke, 2002). With reduced intensity of cattle grazing not only the taller sward height itself could be beneficial for butterflies but also the more heterogeneous sward structure created by the animals.

In this study, the importance of grazing intensity on butterfly diversity in the long term has been investigated, as well as other factors (flower head abundance, sward surface height and heterogeneity) that have the potential of influencing butterfly assemblages in the shorter term.

# Materials and methods

The study was carried out as a randomized block experiment with three replications on a mesophile permanent grassland (plant-association *Lolio-Cynosuretum* with main species *Lolium perenne*, *Dactylis glomerata* and *Festuca rubra*, moderately species rich) in the Solling Uplands, Germany, with Simmental cattle. The factor grazing intensity was adjusted in a put-and-take-system using target compressed sward heights measured biweekly (moderate: 6 cm, lenient: 12 cm, very lenient: 18 cm). The extensive grazing experiment was established in 2002 with the very lenient treatment set up in 2005; for site description, productivity and forage quality details see Isselstein *et al.* (2007).

Butterflies were counted and identified to species level on three fixed 50 m-transects per paddock. Butterfly monitoring consisted of 10 counts per year recorded as described in Wallis de Vries *et al.* (2007). In this paper, we present the results of three summer counts (June/ July, July, August/September) for the years 2003, 2004, 2010 and 2011. In 2011, sward surface height was measured (using the Bircham sward-stick) and flower head abundance (cover of flower heads in 30 cm-circles) was estimated at 100 points along the middle transect line per paddock. For the analysis, mean values of sward surface height and flower head abundance per paddock were taken. Sward structural heterogeneity per paddock was calculated using the coefficient of variation of sward surface height measurements. For statistical analysis, counted species and individuals were pooled over the three occasions per year. Mixed models for the response variables species richness and abundance were carried out using grazing intensity, year (as a nominal variable) and their interaction as fixed factors with repeated measurements over years at the paddock scale. A separate but analogous analysis was made for the years 2010 and 2011 to account for the extra treatment. For 2011, multiple regression models were carried out with grazing intensity, sward surface height, sward height heterogeneity, and flower-head abundance. Model simplification was carried out on all models based on AIC (Akaike information criterion). All statistics were done

#### **Results and discussion**

with R 2.11.1 (R Development Core Team, 2010).

Over all years, more butterfly species were found on lenient grazing than on moderate grazing (P = 0.022, Figure 1a), but there was no significant effect on abundance (P = 0.133, Figure 1b). No interaction between grazing intensity and years was found, neither regarding species richness (P = 0.065) nor abundance (P = 0.234). Both response variables were year-dependent (species richness: P < 0.001, abundance: P = 0.004). In the separate models for the last two years, very lenient grazing showed an increased species richness (P = 0.024) and abundance (P = 0.006) compared with the moderate treatment. The difference between lenient and very lenient grazing in abundance was non-significant (P = 0.124), but there were slightly more species counted on lenient than on very lenient grazing factor was the only term remaining in the model. The abundance model of the last two years displayed a significant



Figure 1. a) Butterfly species richness (number of species) and b) abundance (counts of individuals) at different grazing intensities. Moderate and lenient grazing have been in place since 2002, very lenient grazing since 2005. Error bars show standard deviations. Statistical differences are described in the text

year effect (P = 0.013), but no interaction between year and grazing intensity (P = 0.133). Factor analysis by multiple regression models for the year 2011 emphasized the importance of grazing treatments on species richness (Table 1). Furthermore, flower-head abundance contributed significantly to the model.

Considering butterfly abundance in 2011, sward height heterogeneity remained in the model, even though not significant (Table 1). This was also true for the factors grazing

intensity and flowers percentage, so that the model on the basis of the given variables could not explain the observed abundance patterns sufficiently. Maybe the allowance of a more precise butterfly – plant species identity relationship would be worthwhile in this context.

	Species ri	chness	Abundance				
F-value	12.9	8	4.40				
$R^2$	0.82	2	0.63	0.63			
P-value	0.00	9	0.090				
	F-value P-value		F-value	P-value			
Grazing intensity	15.74	0.007	5.87	0.065			
Flower abundance	7.45	0.041	3.14	0.151			
Sward surface height	_	_	_	_			
Sward heterogeneity	-	_	2.74	0.173			

Table 1. Results of multiple regression models of species richness and abundance of butterflies in 2011. Empty cells indicate term elimination in model simplification using AIC

In summary, butterflies benefitted from less intensive grazing, which is in line with many other studies (e.g. Kruess and Tscharntke, 2002; Dumont *et al.*, 2009). The increasing differences with time between moderate and lenient treatments for both species richness and abundance also suggest that uniform long-term management provides even better conditions for butterfly protection.

Interestingly, the most extensively managed paddocks could not offer better opportunities for butterfly individuals than the lenient ones and they were even less attractive for butterfly species. This points to a unimodal relationship, which was also proposed by Pöyry *et al.* (2004).

#### Conclusions

This study indicates that an unaltered lenient grazing management for many years provides increasingly better conditions for butterfly diversity. This suggests that for biodiversity benefits, and particularly for butterflies, farmers should plan many years in advance to let grazing dynamics develop. However, aiming at butterfly protection does not mean to extensify pasture use to a maximum, as the smallest grazing intensity did not prove to create the most attractive conditions for butterflies. These findings indicate a unimodal relationship between butterfly diversity and grazing intensity, providing good chances for combining biodiversity outcome and farmers' interests.

#### References

Dumont B., Farruggia A., Garel J.-P., Bachelard P., Boitier E. and Frain M. (2009) How does grazing intensity influence the diversity of plants and insects in a species-rich upland grassland on basalt soils? *Grass and Forage Science* 64, 92–105.

Isselstein J., Griffith B.A., Pradel P. and Venerus S. (2007) Effects of livestock breed and grazing intensity on biodiversity and production in grazing systems. 1. Nutritive value of herbage and livestock performance. *Grass and Forage Science* 62, 145–158.

Kruess A. and Tscharntke T. (2002) Grazing intensity and the diversity of grasshoppers, butterflies and trap-nesting bees and wasps. *Conservation Biology* 16, 1570–1580.

Pöyry J., Lindgren S., Salminen J. and Kuussaari M. (2004) Restoration of butterfly and moth communities in semi-natural grasslands by cattle grazing. *Ecological Applications* 14, 1656–1670.

Van Swaay C., Warren N. and Loïs G. (2006) Biotope use and trends of European butterflies. *Journal of insect conservation* 10, 189–209.

Wallis de Vries M.F., Parkinson A.E., Dulphy J.P., Sayer M. and Diana E. (2007) Effects of livestock breed and grazing intensity on biodiversity and production in grazing systems. 4. Effects on animal diversity. *Grass and Forage Science* 62, 185–197.

# *Ex situ* evaluation of forage yield components and forage yields in wild populations of French vetch (*Vicia serratifolia* Jacq.) from Serbia

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# Abstract

Vetches present a good potential for becoming forage crops, and the aim of this study was to evaluate forage yield components and forage yields in the wild populations of French vetch (*Vicia serratifolia* Jacq.) in Serbia. A small-plot trial was carried out in 2010 and 2011 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi, including twelve wild populations of French vetch collected at diverse locations at the mountain Fruška Gora in Serbia. The population VS 05 had the highest two-year average forage yields, namely 41.6 t ha<sup>-1</sup> of green forage (9.6 t ha<sup>-1</sup> of forage dry matter), while the population VS 04 had the lowest two-year average forage yields, namely 9.0 t ha<sup>-1</sup> of green forage (2.1 t ha<sup>-1</sup> of forage dry matter). Some of the populations tested produced yields similar to those of faba bean (*V. faba* L.) and higher than Narbonne vetch (*V. narbonensis* L.) in the same agroecological conditions. They confirm their considerable potential for forage production in temperate regions such as the northern Balkans.

Keywords: *ex situ* evaluation, forage yield components, forage dry matter yield, French vetch, green forage yield, *Vicia serratifolia* 

# Introduction

Vetches (*Vicia* spp.) are widely recognised as valuable components of natural grasslands and as forage crops of high quality. The wild and agricultural flora of Serbia records about 30 vetch species, with common (*V. sativa* L.), Hungarian (*V. pannonica* Crantz) and hairy (*V. villosa* Roth) vetches as the economically most important (Mikić *et al.*, 2011).

A long-term concerted action between the Institute of Field and Vegetable Crops and Faculty of Agriculture in Novi Sad is aimed at collecting, preserving, maintaining, characterising and evaluating the wild populations and local landraces of diverse vetch species in Serbia (Mikić *et al.*, 2009b). Narbonne vetch (*Vicia narbonensis* L.) and French vetch (*Vicia serratifolia* Jacq.) are the closest botanical relatives of faba bean (*Vicia faba* L.) and thus may have a potential agronomic importance (Berger *et al.*, 2003; Kendir *et al.*, 2009). The aim of the study was to evaluate forage yield components and forage yields in the wild populations of French vetch of Serbian origin.

# Materials and methods

A small-plot trial was carried out in 2010 and 2011 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi. It included twelve wild populations of French vetch collected at diverse locations at the mountain Fruška Gora in 2009.

In both trial seasons all twelve populations were sown in early March, at a rate of 150 viable seeds  $m^{-2}$  (Mihailović *et al.*, 2005), a plot size of 5  $m^2$  and three replicates. Each population was cut in the stages of full flowering and first pods, as a balance between forage yield and quality, namely in the first half of June in both years.

Plant height (cm), numbers of stems and lateral branches (plant<sup>-1</sup>), number of internodes (plant<sup>-1</sup>), number of green leaves (plant<sup>-1</sup>), leaf proportion and green forage yield (g plant<sup>-1</sup>) were determined from the plant samples taken before cutting. Green forage yield (t ha<sup>-1</sup>) was measured *in situ* immediately after cutting. Forage dry matter yield (g plant<sup>-1</sup> and t ha<sup>-1</sup>) was determined on the basis of a ratio between masses of green forage samples after and before the drying at room temperature. Forage dry matter proportion was calculated as a ratio between forage dry matter yield and green matter yield.

Results were processed by analysis of variance (ANOVA) applying the Least Significant Difference (LSD) test using the computer software MSTAT-C.

## **Results and discussion**

There were significant differences among the average values of all monitored forage yield components (Table 1). The average plant height varied between 63 cm in the population VS 08 and 171 cm in the population VS 02. The population VS 09 had the greatest average number of both internodes (43 plant<sup>-1</sup>) and green leaves (31 plant<sup>-1</sup>), while the population VS 08 had the smallest average number of both internodes (10 plant<sup>-1</sup>) and green leaves (10 plant<sup>-1</sup>). The proportion of leaf in the total green forage yield per plant ranged from 0.37 in the population VS 08.

The population VS 05 had the highest two-year average forage yields, namely 29.30 g plant<sup>-1</sup> and 41.6 t ha<sup>-1</sup> of green forage and 6.74 g plant<sup>-1</sup> and 9.6 t ha<sup>-1</sup> of forage dry matter (Table 2). On the other hand, the population VS 04 had the lowest two-year average forage yields, namely 6.12 g plant<sup>-1</sup> and 9.0 t ha<sup>-1</sup> of green forage and 1.44 g plant<sup>-1</sup> and 2.1 t ha<sup>-1</sup> of forage dry matter. Some of the tested wild populations of French vetch produced forage yields at the same level as those of faba bean (*V. faba* L.) or higher than in Narbonne vetch (*V. narbonensis* L.) in the same agroecological conditions (Mikić *et al.*, 2009a; Mihailović *et al.*, 2010). The population VS 08 had the highest forage dry matter proportion (0.28).

Population	Plant height (cm)	Number of stems (plant <sup>-1</sup> )	Number of inter- nodes (plant <sup>-1</sup> )	Number of green leaves (plant <sup>-1</sup> )	Leaf proportion
VS 01	130	1	20	15	0.45
VS 02	171	1	33	23	0.45
VS 03	85	1	17	17	0.54
VS 04	140	1	20	14	0.37
VS 05	147	1	23	15	0.55
VS 06	72	1	13	13	0.57
VS 07	130	1	22	12	0.48
VS 08	63	1	10	10	0.63
VS 09	126	2	43	31	0.51
VS 10	132	1	19	17	0.50
VS 11	120	1	26	21	0.53
VS 12	87	2	29	27	0.56
Mean	117	1	23	18	0.51
LSD	34	0.5	12	10	0.11

Table 1. Average values of forage yield component in wild populations of French vetch for the years of 2010 and 2011 at Rimski Šančevi

Population	Green forage yield (g plant <sup>-1</sup> )	Green forage yield (t ha <sup>-1</sup> )	Forage dry matter yield (g plant <sup>-1</sup> )	Forage dry matter yield (t ha <sup>-1</sup> )	Forage dry mat- ter proportion
VS 01	18.99	27.2	4.27	6.1	0.23
VS 02	24.74	36.1	5.81	8.5	0.24
VS 03	12.95	18.4	3.24	4.6	0.25
VS 04	6.12	9.0	1.44	2.1	0.24
VS 05	29.30	41.6	6.74	9.6	0.23
VS 06	16.85	24.3	4.04	5.8	0.24
VS 07	22.07	30.7	4.99	6.9	0.23
VS 08	12.07	17.1	3.38	4.8	0.28
VS 09	20.94	29.9	4.82	6.9	0.23
VS 10	16.23	23.9	3.90	5.7	0.24
VS 11	22.53	33.3	5.50	8.1	0.24
VS 12	19.63	29.2	4.65	6.9	0.24
Mean	18.54	26.7	4.40	6.3	0.24
LSD <sub>0.05</sub>	5.67	8.2	1.36	1.9	0.03

Table 2. Average values of forage yield component in wild populations of French vetch for the years of 2010 and 2011 at Rimski Šančevi

## Conclusions

Although still in the initial stages of its evaluation as a potential crop, some of the collected wild populations of French vetch of Serbian origin appear to have a considerable potential for forage production in temperate regions such as the northern Balkans. Further evaluation of this species will target forage quality, with emphasis on the content of crude protein and fibre in forage dry matter, and seed yields, as a basis for possible commercialisation.

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#### References

Berger J.D., Robertson L.D. and Cocks P.S. (2003) Agricultural potential of Mediterranean grain and forage legumes: 2) Anti-nutritional factor concentrations in the genus *Vicia. Genetic Resources and Crop Evolution* 50, 201–212.

Kendir H., Sahin-Demirbag N., Aasim M. and Khawar K.M. (2009) *In vitro* plant regeneration from Turkish Narbon Vetch (*Vicia narbonensis* L. var. *narbonensis* L.). *African Journal of Biotechnology* 8, 614–618.

Mihailović V., Mikić A., Karagić Đ., Pataki I. and Krstić Đ. (2005) Genetic variability of yield and its components in spring vetch cultivars. *Grassland Science in Europe* 10, 303–306.

Mihailović V., Mikić A., Vasić M., Ćupina B., Đurić B., Duc G., Stoddard F. L. and Hauptvogel P. (2010) Neglected legume crops of Serbia – Faba bean (*Vicia faba*). *Ratarstvo i povrtarstvo / Field and Vegetable Crops Research* 47, 27–32.

Mikić A., Mihailović V., Ćupina B., Vishyakova M., Vasić M., Đorđević V. and Perić V. (2009a) Forage and grain yields in the VIR accessions of narbonne vetch *Vicia narbonensis* in the conditions of Serbia. *Bulletin of Applied Botany, of Genetics and Plant Breeding* 188, 185–188.

Mikić A., Mihailović V., Hauptvogel P., Ćupina B., Petrović M., Krstić D., Jovičić D., Milošević B. and Hauptvogel R. (2009b) Wild populations of vetches (*Vicia*) as forage and green manure crops for temperate regions. *Irish Journal of Agricultural and Food Research* 48, 265.

Mikić A., Mihailović V., Ćupina B., Đorđević V., Milić D., Duc G., Stoddard F.L., Lejeune-Hénaut I., Marget P. and Hanocq E. (2011) Achievements in breeding autumn-sown annual legumes for temperate regions with emphasis on the continental Balkans. *Euphytica* 180, 57–67.

# Semi-natural phytocenoses of wet meadows in the Leniwa Notec River valley (Mościckie meadows)

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# Abstract

The investigations were concerned with floristic and site variability of semi-natural associations of wet eutrophic and calciphile meadows from the following alliances: *Calthion palustris, Alopecurion pratensis* and *Filipendulion ulmariae*. Phytosociological studies employed the Braun-Blanquet method; site conditions were assessed on the basis of Ellenberg's F, R and N indicator numbers; moisture conditions were estimated by Oświt's method, and fodder value score according to Filipek. The studies demonstrated that the identified multispecies communities with well-preserved semi-natural vegetation were adapted to sites of varying wetness, were characterised by different yields and fodder value and occupied a high position in the domestic strategy of protection and sustainable utilisation of biologically diversified grasslands.

Keywords: semi-natural phytocenoses of wet meadows, use value number, evaluation category, Ellenberg's indicator numbers

# Introduction

Semi-natural meadow communities in the Leniwa Noteć River valley occupy swampy, frequently inundated areas within the Natura 2000 zone. The Noteć river floods, as well as the frequency of their occurrence, constitute the most important factor affecting the formation and structure changes of plant communities. The above-mentioned changes are frequently of degenerative and regressive character leading to destruction of the primary floristic composition and structure of phytocenoses and are dangerous for them. Human activities, especially the intensity and methods of utilization, should be treated as a factor that significantly transforms the flora of river valleys and sites. The objective of the performed experiments was to determine floristic and site diversity of semi-natural wet meadows in the Leniwa Noteć River valley, as well as their natural-use values.

# Materials and methods

Investigations on the floristic and site variability of semi-natural associations of wet eutrophic and calciphile meadows on Mościckie Meadows situated in the Leniwa Noteć River valley were carried out on an area of 260 ha in 2006–2010. On the basis of 146 phytosociological reléves taken with the assistance of the Braun-Blanquet (1951) method, the authors determined plant systematics and identified syntaxons. The current state of site conditions was calculated on the basis of values of Ellenberg's (1992) indicator numbers: F – moisture content, R – soil reaction and N – nitrogen content in soil. Natural values were calculated employing Oświt's method (1992), while the use value was evaluated according to Filipek (1973). Names of plant communities and species phytosociological rank are given according to the syntaxonomic classification for Wielkopolska published by Brzeg and Wojterska (2001). Plant names – Polish and Latin – are given after Mirek *et al.* (2003).

# Results

#### Phytosociological diagnosis

Using the syntaxonomic classification for Wielkopolska Brzeg and Wojterska (2001), phytocenoses of semi-natural Mościckie Meadows in the Leniwa Noteć River valley were found to comprise meadows or flowery swards containing numerous dicotyledonous species (Grzelak and Bocian, 2006). They belong to the *Molinio-Arrhenatheretea* R. Tx. 1937 em. 1970 class of the *Molinietalia* W. Koch 1926 order. Meadow flora belonged to three associations of marigold meadows from the *Calthion* R. Tx. 1937 alliance: brook thistle meadow – *Angelico-Cirsietum oleracei* R. Tx. 1937 em. 1947 (distinctly dominant with respect to the area), wood bulrush meadows – *Scirpetum sylvatici* Ralski 1931 and hairgrass meadows – *Stellario-Deschampsietum* Freitag. *Alopecuretum pratensis* (Regal, 1925), Steffen 1931 association was identified in the *Alopecurion pratensis* Pass.1964 alliance, while in the *Filipendulion ulmariae* Segal 1966 (= *Filipendulo-Petasition* Br.-Bl. 1947 p.p.) alliance – the *Filipendulo-Geranietum* W. Koch 1926 association was determined.

Within the area of diagnosed phytocenoses, 48 species from the *Molinio-Arrhenatheretea* class were identified. The following numerous diagnostic species occurred in 100% phytosociological reléves: cabbage thistle (*Cirsium oleraceum*), water avens (*Geum rivale*), greater bird's-foot trefoil (*Lotus uliginosus*), marsh thistle (*Cirsium palustre*), bulrush (*Scirpus sylvaticus*), kingcup (*Caltha palustris*), ragged robin (*Lychnis flos-cuculi*) and common bistort (*Polygonum bistorta*). The following other taxons of the diagnostic group were also present outside reléve patches: marsh cranesbill (*Geranium palustre*), water forget-me-not (*Myosotis palustris*) and garden valerian (*Valeriana officinalis*).

# Natural values and productivity

On the basis of species occurring in plant communities (Oświt, 2001), the identified associations are characterized by moderate natural values of IV<sup>th</sup> valorization class, with the exception of the *Angelico-Cirsietum oleracei* association (class V; Table 1). With respect to their utilization, semi-natural meadows are usually cut once or twice a year. Hay yields vary and are estimated to range from 1.5-3.0 t ha<sup>-1</sup> in the *Filipendulo-Geranietum* association to 6.0-8.0 t ha<sup>-1</sup> in the *Alopecuretum pratensis* association. Fodder value score (FVS) is estimated at 2.2 to 5.6 (Table 1).

Evalua-	Natural qualities	Hay yield	UVN	Sward
tion category		t IIa		value
V	Moderately high	4.2-5.8	4.1	good
IV	Moderate	3.8-5.6	3.2	poor
IV	Moderate	3.1-3.6	3.1	poor
IV	Moderate	6.0-8.0	5.6	good
IV	Moderate	1.5-3.0	2.2	poor
	Evalua- tion category V IV IV IV IV	Evalua- tion categoryNatural qualitiesVModerately high ModerateIVModerateIVModerateIVModerateIVModerate	Evalua- tion categoryNatural qualitiesHay yield t ha-1V IVModerately high Moderate4.2–5.8 3.8–5.6IVModerate3.1–3.6IVModerate6.0–8.0IVModerate1.5–3.0	Evalua- tion categoryNatural qualitiesHay yield t ha <sup>-1</sup> UVN t ha <sup>-1</sup> V IVModerately high Moderate4.2–5.84.1IVModerate3.8–5.63.2IVModerate3.1–3.63.1IVModerate6.0–8.05.6IVModerate1.5–3.02.2

Table 1. Natural qualities, hay yields, utilisation value number (UVN-index) and sward value of plant communities

# Evaluation of site conditions

The identified communities were characterized by high moisture content or marshy conditions, as indicated by the Ellenberg's index (Table 2). The examined area is flooded or inundated, even in summer.

Plant community	Watland habitat	]	Ellenberg index*				
	wetianu nabitat	F	R	Ν			
Calthion							
Angelico-Cirsietum oleracei	Strongly moist	5.8	6.7	6.1			
Scirpetum silvatici	Swampy	7.0	6.2	6.5			
Stellario palustris-Deschampsietum							
caespitosae	Strongly moist	6.9	6.4	4.5			
Alopecurion pratensis							
Alopecuretum pratensis	Strongly moist	7.9	5.5	5.2			
Filipendulion ulmariae							
Filipendulo-Geranietum	Strongly moist	8.0	6.9	3.8			

Table 2. Site conditions determined using the Ellenberg Index

\*Ellenberg's indicator numbers: F – moisture, R – soil reaction, N – nitrogen content in soil.

#### Conclusions

On the examined area of 260 ha, the following naturally valuable associations from the following alliances were identified: *Calthion palustris*, *Alopecurion pratensis* and *Filipen-dulion ulmariae*. Some of these associations can be found on the list of biotopes which are under legal protection in Poland and appear in the IDS enclosure of Natura 2000. They increase significantly the species diversity, and provide a shelter for many rare and protected plant species, including orchids. Among problems is the disappearance of species characteristic of associations, as well as declining areas of well-developed, typical forms of associations which are being replaced by 'trunked' communities as a result of intensification of utilization, the leaving of mown biomass, or the complete abandonment of utilization.

#### References

Braun-Blanquet J. (1951) Pflanzensoziologie. Springer Verlag, Wien.

Brzeg A. and Wojterska M. (2001) Vegetation associations in Wielkopolska, their state of recognition and vulerability. In: Wojterska M. (ed.). *Vegetation cover of Wielkopolska and Southern Pomeranian Lake District*. Guide of field sessions of the 52nd Meeting of PTB, 24–28 September 2001, Bogucki Wydawnictwo Naukowe, Poznań, pp. 39–110 (in Polish).

Ellenberg H. (1992) Zeigerwerte von Pflanzen In Mitteleuropa. Scripta Geobotanica 18, 5-528.

Filipek M. (1973) The proposal for the classification of meadow and pasture plants on the basis. *Postępy Nauk Rolniczych* 4, 59–68 (in Polish).

Grzelak M. and Bocian T. (2006) Geobotanical diversity of semi-natural communities of the "Bystra" Noteć valley and their role in the landscape. *Annales UMCS* 61, sectio E, 257–266 (in Polish).

Mirek Z., Piękoś-Mirkowa H., Zając A. and Zając M. (2003) *Flowering plants and pteridiophytes of Poland*. A checklist. Instytut Botaniki PAN, Kraków, pp. 442 (in Polish).

Oświt J. (2000) The method of valuation of natural wetlands and the results of its application to selected objects. IMUZ Falenty, 3–32 (in Polish).

# The effect of human disturbance on the occurrence and propagation of common ragweed (*Ambrosia artemisiifolia* L.) in grasslands with different management types

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# Abstract

The occurrence and spread of common ragweed in grasslands of southwest Hungary with different management was investigated. All grassland sites have been used for deer grazing. On each site, plant species were recorded and their cover was estimated over three successive years. On all sites two plots were sampled: a control plot with common management measures and a plot situated inside the disturbance area. All disturbance effects increased the cover of common ragweed compared to the control plots, varying between 1% and 90%. The ragweed cover increase showed a close link with the intensity of disturbance imposed by management. Minimal human disturbance, like tree cutting in the border of the stand, resulted a small increase (1–5%) of ragweed cover, while a more intensive manure and silage deposit resulted a higher ragweed cover increase (25–90%). Another correlation was observed between the time passed after a particular human activity and the ragweed coverage decrease in all habitats in which the human disturbance was stopped in the first or second year.

Keywords: common ragweed, spreading, human activities, grassland management

#### Introduction

Common ragweed (*Ambrosia artemisiifolia* L.) is one of the most important alien weed species in Hungary. This native American plant was introduced in Western Europe at the end of the 19<sup>th</sup> century (Gladieux *et al.*, 2011), reaching Eastern Europe at the beginning of the 20<sup>th</sup> century, and is now invading the temperate regions of Europe and Asia (Kazinczi *et al.*, 2008). Its spreading is more and more intensive and causes serious economic losses in crop yields (Protopopova *et al.*, 2006). This weed is also responsible for human allergic problems, due to its enormous pollen production (D'Amato *et al.*, 2007). In Hungary, the spreading of the common ragweed in arable fields was investigated (Pinke *et al.*, 2011), but until now no information has been available about its spreading in semi-natural grassland habitats. Our hypothesis was that all human interventions which are not common grassland management measures increase the cover of common ragweed.

#### Materials and methods

The occurrence and propagation of common ragweed was investigated in Bőszénfa (Somogy county, Southwest Hungary) in natural and semi-natural grasslands, as well as in grass seeding sites used for deer pasturing, in order to examine the effects of different human activities (grass fertilisation, pipeline construction, manure application, deer feeding, silage deposits) and the effect of grassland seeding on common ragweed appearance and spreading. In each location, control plots (undisturbed plots with common ragweed coverage  $\leq 1\%$ ) and affected sample plots were examined simultaneously in three successive years, with a summer and an autumn sampling per year. The plots were chosen randomly from

each homogenous site. The repeated samplings were done exactly on the same plots, and cover values were made for the three years together per plot.

The surveyed plots were:

- S1 Lolium perenne-dominated natural grassland (control and tree-cutting expansion sites);
- S2 *Lolium perenne*-dominated natural grassland (control and pipeline construction affected sites);
- S3 *Festuca pratensis-Lolium perenne* dominated semi-natural grassland (control and manure heap deposit-affected sites);
- S4 *Festuca arundinacea*-dominated semi-natural grassland (control and manure heap deposit-affected sites);
- S5 *Lolium perenne*-dominated semi-natural grassland (control and deer feeding affected sites);
- S6 Lolium perenne-dominated natural grassland (control and silage-deposit affected sites);
- S7 Trifolium pratense-Lolium perenne seeding (successful seeding in 2007);
- S8 Trifolium pratense-Lolium perenne seeding (successful seeding in 2009);
- S9 *Trifolium pratense-Lolium perenne* seeding (unsuccessful seeding in 2007, re-seeding in 2009).

For the S6, S7, S8 and S9 samples only one control plot was taken, because the silage deposits and the seeding have changed the species composition of the whole area of the affected sites. In this case, the control plot represents the original plant cover in the vicinity of these four affected sites. In all  $2\times2$  m plots, a plant species list was recorded and cover percentages were estimated for each species. In all control plots the ragweed cover was under 1%. To establish the connection between the cover percentages of the plots, correspondence analysis (CCA) was used. The data analysis was made with NuCoSA software (Tóthmérész, 1993).

#### **Results and discussion**

All affected plots showed higher cover of common ragweed compared with the undisturbed plot at the same site. The measure of common ragweed cover increase varied between 1 and 90%, depending on the nature of human activity. The activities causing the lowest cover increase were the expansion of tree-cutting (1% ragweed cover increase; S1 plot) and the water pipeline construction (5% increase; S2 plot). Higher values of ragweed cover were measured in the case of manure deposited in the vicinity of the sample plot (25–50%; S3 and S4 plots) and deer feeding (75%; S5 plot). The highest ragweed cover was observed in the plot near the silage deposit, as compared with the undisturbed control plot (90%; S6 plot). Grass seedings also showed higher values of common ragweed cover, but the measure depended on the seeding success. In successfully seeded plots the cover was 5% (S7 plot), while in the unsuccessfully seeded plot it was 50% (S9 plot).

Another connection was observed between the seeding date and the ragweed cover values. The successful seeding made in 2007 showed 5% common ragweed cover, whereas the successful seeding made in 2009 showed 50%.

Figure 1 shows the distribution of all sample plots. The 1<sup>st</sup> axis corresponds with the common ragweed cover increase. On the left side were grouped the control plots (except S5 stand), and on the right side the affected plots with higher ragweed coverage. The control S5 plot differs for all others, caused by the presence of shrubs, missing in the other stands. The 2<sup>nd</sup> axis corresponds with the type of human activity. In the lower part were grouped the more affected seeding plots and the silage deposit-area stand, followed by the manure deposit and deer feeding plots. In the upper part the majority of the control plots were grouped.



Figure 1. Correspondence Analysis (based on Matushita comparative function) of the examined plots. 1 = S1 control; 2 = S1 tree cutting plot; 3 = S2 control; 4 = S2 pipeline construction plot; 5 = S3 control; 6 = S3 manure deposit; 7 = S4 control; 8 = S4 manure deposit; 9 = S5 control; 10 = S5 deer feeding plot; 11 = S6,7,8,9 control; 12 = S6 silage deposit; 13 = S7 2007 seeding; 14 = S8 2009 seeding; 15 = S9 re-seeding

#### Conclusions

In parallel with the increasing measure of human disturbance, the appearance and spread of common ragweed can increase seriously in grasslands and on grass seeding sites, and in this way the invasive success of this very dangerous weed has been promoted in Hungary. In order to inhibit the spreading of *Ambrosia artemisiifolia*, thoroughly planned land use and establishment of grasslands on poorer soil quality croplands – where the spreading of common ragweed is of significance – are necessary.

#### References

D'Amato G., Cecchi L., Bonini S., Nunes C., Annesi-Maesano I., Behrendt H., Liccardi G., Popov T. and Van Cauwenberge P. (2007) Allergenic pollen and pollen Allergy in Europe. *Allergy* 62, 976–990.

Gladieux P., Giraud T., Kiss L., Genton B.J., Jonot O. and Shykoff J.A. (2011) Distinct invasion sources of common ragweed (*Ambrosia artemisiifolia*) in Eastern and Western Europe. *Biological Invasions* 13, 933–944. Kazinczi G., Béres I., Novák R., Bíró K. and Pathy Z. (2008) Common ragweed (*Ambrosia artemisiifolia*): a review with special regards to the results in Hungary. I. Taxonomy, origin and distribution, morphology, life cycle and reproduction strategy. *Herbologia* 9/1, 55–91.

Pinke Gy., Karácsony P., Czúcz B. and Botta-Dukát Z. (2011) Environmental and land-use variables determining the abundance of *Ambrosia artemisiifolia* in arable fields in Hungary. *Preslia* 83, 219–235.

Protopopova V.V., Shevera M.V. and Mosyakin S.L. (2006) Deliberate and unintentional introduction of invasive weeds: A case study of the alien flora of Ukraine. *Euphytica* 148, 17–33.

Tóthmérész B. (1993) NuCoSA 1.0: Number Cruncher for Community Studies and other Ecological Applications. *Abstracta Botanica* 17, 283–287.

# Habitat modelling with high resolution in an agricultural landscape

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# Abstract

A habitat model was developed to describe the relationship between landscape features, agricultural management and the distribution of five plant species. The model was based on data from 1028 randomly chosen  $10 \text{ m} \times 10 \text{ m}$  plots. This data set was combined with data on environmental variables in  $10 \text{ m} \times 10 \text{ m}$  cells in a regular grid covering the study area (5 km × 6 km). Environmental variables were obtained from data that already exists in digital form, e.g. topographic variables and vegetation types, or derived from aerial photos. The environmental variables included in the model are vegetation type, slope, aspect and concavity of the terrain and a wetness index derived from a digital elevation model. The five species appeared in a wide range of vegetation types but semi-natural grasslands without use of fertilizer were by far the most important habitat. The distribution within semi-natural grasslands and pastures are highly influenced by topography. For example, *Knautia arvensis* had a higher probability of occurrence in south-facing habitats with slightly convex terrain. Some of the species occurred regularly in forests and this might be a result of encroachment of abandoned grasslands. This will be further investigated by including historical vegetation structure and land-use derived from aerial photos in the model.

Keywords: habitat modelling, landscape change, semi-natural grassland, species distribution

# Introduction

The study of species response to ecological gradients and land-use is an important part of ecology and conservation biology. In the last 10 years or so, a number of novel methods have been used to describe and predict how ecological gradients and land-use influence the distribution of wildlife species in real landscapes (Franklin, 2009; Dormann, 2011). The main objective of the study is to relate the fine-scale distribution of the five study species to landscape characteristics and agricultural land-use.

# Methods and data

In this study, we have used boosted regression trees (BRT) to describe the distribution of five plant species characteristic of semi-natural grasslands in Norway (Trøndelag region). The five study species are: *Campanula rotundifolia, Knautia arvensis, Leontodon autumnalis, Pimpinella saxifraga* and *Ranunculus auricomus*. The study uses a methodological approach outlined by Elith *et al.* (2008). The occurrence of the five study species was recorded as presence-absence in 1028 randomly chosen  $10 \times 10$  m plots within a landscape of approximately 5 km × 6 km. This constitutes the training data for the model. Environmental data (also referred to as background data, Dormann, 2011) were obtained by creating a raster map in ArcGIS software where each 10 m × 10 m pixel corresponds to the regular grid used to position the sampling plots in the field. The environmental data contained the variables habitat type<sup>1</sup>, slope, aspect, concavity of the terrain and a wetness index derived from

<sup>&</sup>lt;sup>1</sup>Habitat type outlined from the AR5 mapping system used in Norway.

a digital elevation model. The eight habitat types defined within the study area were included in the model as a set of dummy variables (although categorical variables other than binary variables can be included in BRT models, Hastie *et al.*, 2009). This was done to ease the interpretation of the model. The study also includes an independent evaluation data set used to evaluate the predictive ability/performance of the different models. A similar sampling design was used for collecting the evaluation data as for the learning data. The BRT models were built using the R computing environment (R Development Core Team, 2011) and the package dismo (Hijmans *et al.*, 2011). Area under the curve (AUC) was used as a measure of predictive performance.

#### **Results and discussion**

Semi-natural grasslands and permanent pastures were the most important habitats for all species. The variable coding for these habitat types came out as the most important predictor for all five study species. Nevertheless, some of the species also had relatively high probability of presence in forest but this was highly influenced by forest type. To give an example, the probability of having *Campanula rotundifolia* in a pixel was positively influenced by the habitat type deciduous forest and negatively influenced by mixed forest. The small-scaled distribution of the study species within semi-natural grasslands and pastures was highly influenced by environmental variables which describe the shape of the terrain. Terrain shape also influenced the probability of *Campanula rotundifolia* in deciduous forests. *Campanula rotundifolia* had a higher probability of occurrence in very steep deciduous forests with highly convex terrain. Terrain variables such as slope and concavity are likely to have a high influence on water availability, which in turn influences other biotic and abiotic variables. In forests, terrain shape can influence the availability of light at the forest floor.



Figure 1. This 3D plot is an example of how boosted regression trees can be used to model the predicted probability of occurrence of a species in response to environmental variables. The plot shows the relationship between predicted probability of *Campanula rotundifolia* and the two predictors, aspect (in degrees, 180 degrees being south) and slope

Aspect, i.e. the 'southness' of the terrain, was an important predictor variable in *Knautia* arvensis and *Pimpinella saxifraga*, but also influenced probability of presence of *Ranunculus* auricomus, Leontodon autumnalis and Campanula rotundifolia (Figure 1), North-facing grasslands in the study area had considerably lower abundance of the five study species and appeared to be more grass-dominated. In several of the species, there was a strong interaction between aspect and one or more of the variables describing terrain shape. For example, *Knautia arvensis* had a higher probability of occurrence in south-facing habitats with slightly convex terrain.

For all species but one the AUC values are in the range 0.8–0.93, which indicate high predictive ability of the models (Hosmer and Lemeshow 2000). *Leontodon autumnalis* had an AUC of 0.695, which can be considered to be an intermediate predicate performance. Possible explanations for the lower predictive performance of the model for *Leontodon autumnalis* are a broader ecological niche and higher occurrence in grasslands on arable land. In the field we observed that the occurrence of *Leontodon autumnalis* on more productive grasslands varied with agricultural management. The lack of variables which describe agricultural management in the model can potentially be an additional reason for the lower predictive power in this species. The models for all species can probably be improved by including predictor variables which describe present and historical agricultural management. This will be focused in the further development of the models.

#### References

Dormann C.F. (2011) Modelling Species' Distributions. – In: Joop et al. (eds). Modelling Complex Ecological Dynamics. Springer, Berlin, pp. 179-196.

Elith J., Leathwick J.R. and Hastie T. (2008) A working guide to boosted regression trees. *Journal of Ecology* 77, 802-813.

Franklin J. (2009) *Mapping species distributions: spatial inference and prediction*. Cambridge University Press, Cambridge, UK.

Hastie T., Tibshirani R. and Friedman J.H. (2009) The elements of statistical learning: data mining, inference, and prediction, 2nd edn. Springer, New York.

Hijmans R., Phillips S., Leathwick J. and Elith J. (2011) *Package 'dismo': Species distribution modeling*. R package version 0.7-11. Freely available from http://cran.r-project.org/package=dismo

Hosmer D.W. and Lemeshow S. (2000) *Applied Logistic Regression*, 2<sup>nd</sup> edition. John Wiley & Sons, New York. R Development Core Team (2011) *R*: *A language and environment for statistical computing*. *Version 2.13*. R Foundation for Statistical Computing, Vienna, Austria.

# The restoration of bog ecosystems on out-of-use meadows

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# Abstract

Ecological studies were carried out in Central Poland in bog areas reclaimed in the 1960s. Communities of the class *Molinio-Arrhenatheretea* developed there after land management. Less-intensive land use and conservation of reclamation facilities in the beginning of the 1990s changed the habitats and grassland communities. An increase of soil moisture and recovery of bog-forming processes were observed in some habitats. Herb and hair grass communities started to develop in part of these habitats. Secondarily bogged sites were colonised by taxa and vegetation communities similar to those present there previously.

Keywords: habitat and grassland community, transformations, secondarily bogged meadows

# Introduction

Most meadow communities in Poland developed after reclamation and management of former bog areas. These communities belonged mainly to the class *Molinio-Arrhenatheretea*. Less-intensive land use and conservation of reclamation facilities in the beginning of the 1990s initiated various changes in habitats and grassland communities. Part of the sites experienced an elevation of the ground water table. Degradation and the development of herb and hair grass communities were frequently observed, and in wet sites plant succession led to the development of more diverse communities of higher ecological value. Taxa of the classes present there prior to land reclamation started to recover. In secondarily bogged habitats, grassland communities changed their taxonomic affiliation and turned from the class *Molinio-Arrhenatheretea* to the class *Scheuchzerio-Caricetea nigrae* or *Phragmitetea*. A high level of ground water in unused meadows hampered the development of trees and shrubs; nevertheless, endangered and protected species were found sporadically. The aim of this study was to demonstrate changes in soil moisture, habitat fertility and grassland communities between the years 1991 and 2009.

#### Materials and methods

Studies were carried out in Central Poland in the years 1991–2009 in reclaimed grasslands. Nine representative sites on organic soils were selected. Soil type (with the soil pit method), the content of P and K in the 0–20 cm soil layer (in 0.5 M HCl soil extract), ground water level (during the first and second cuts) and botanical composition (with the Klapp's method) were determined.

#### **Results and discussion**

Analysed sites had moderately and poorly decomposed peat-muck soils (Mt) and mineralmuck soils (Mr). Organic matter content ranged from 50 to 80% in the former and from 20 to 55% in the latter (Kozłowska and Frąckowiak, 2011). In the beginning of the study, the soils showed high and very high P content and very low K content. In the year 2010 the content of K and P decreased in most sites except in those flooded by river waters (sites 2, 7, 9) and in utilised grasslands where the P content was still very high and the K content was of medium value as in utilised site 1 (Table 1).

Elamant	Derr en 1)	Content	Years and sites					
Element	Kange	mg 100 g <sup>-1</sup> DW	1992	2010				
	very low	<17.5		3, 5, 6, 8				
D	low	17.5-26.2	8					
Г	high	35.1-52.5	4, 5, 9	4				
	very high	>52.5	1, 2, 3, 6, 7	1, 2, 7, 9				
V	very low	<25	3, 4, 5, 6, 7, 8, 9	2, 3, 4, 5, 6, 7, 8, 9				
<u>к</u>	medium	51-75	1, 2	1				

Table 1. Ranges of the content of soluble K and P forms in the 0–20 cm soil layer (the years 1992 and 2010)

<sup>1)</sup>- acc. to Sapek and Sapek (1997).

Neglected conservation of reclamation facilities had already resulted in a large differentiation of ground water levels among cuts and sites at the start of this study in 1991. During the second cut the water was above soil surface (at site 5), in the root zone or just beneath it (sites 8 and 6), and its deepest level was 40–45 cm (sites 2 and 3) (Figure 1). Large variations at a gradual increase of the ground water level were observed in the next years in all sites. In site 1 it was always below 30 cm and in others it was usually higher, often reaching the root zone or soil surface. In the sites with higher level of ground water (sites 5, 6, 7) the process of bogging returned spontaneously.





Figure 1. Ground water level in the 1<sup>st</sup> and 2<sup>nd</sup> cut in selected years of the period 1991–2009

Figure 2. Simplified botanical composition of meadow communities in selected sites (2, 4, 5, 7, 8, 9) in the years 1991, 1999, 2009

Until 1991 the grasslands were mown and fertilised. Communities of the class Molinio-Arrhenatheretea developed there. The communities were dominated by grasses of very good or good agricultural value. The first site abandoned in 1991 was site 1, and utilisation of others was abandoned in subsequent years. Now, only site 1 is utilised. A lack of conservation of reclamation facilities resulted in the increase of ground water table. The share of medium and poor grasses (mainly Deschampsia caespitosa) and/or herbs, weeds and sedges increased in studied communities (Figure 2). Initially, an increase of soil moisture, at large variability of ground water levels, increased the number of species in communities (sites 3, 4, 7), mainly of herb and weed species. Further elevation of ground water level, its maintenance in the root zone and flooding decreased the number of species and resulted in the domination of sedges sites 5 and 7). Carex riparia dominated in flooded fertile sites (site 7), at smaller fertility the dominants were C. gracilis (site 6) or C. nigra (site 5). According to Peeters et al. (1994) the decreasing content of available P is the reason for decreased number of species in the community. In our studies, ground water level was the driving factor. Taxa from classes present in the study area previously (Traba, 2001) subsequently reappeared and grassland communities changed their taxonomic affiliation. The greatest changes took place in three sites with a high ground water level. Communities of the class Molinio-Arrhenatheretea turned into the class Scheuchzerio-Caricetea nigrae (site 5) or *Phragmitetea* (sites 6 and 7) with a small share of species from the class *Molinio*-Arrhenatheretea, High ground water level limited the development and expansion of trees and shrubs; nevertheless, the endangered and protected species (Comarum palustre L. and Menyanthes trifoliata L.) were rare.

Community	Vaara	Sites									
Community	rears	1	2	3	4	5	6	7	8	9	
Molinio-Arrhenatheretea	2009	24									
Poa pratensis Festuca rubra	1991 1999	27	23 25	9							
	1991								34	20	
Calthion	1999				27	35		38	33	17	
Anielico-Cirsium oleracei	2009 1991						31		30	26	
Deschampsia caespitosa	1991 1999	27		13	21						
Lythro-Filipenduletum	2009 1999	27	22	17	30		29	48			
Magnocaricion	1999 2009					34	34				
Caricetum ripariae	2009							3			
Caricetum nigrae	2009					27					

Table 2. The number of species in grassland communities and the change of taxonomic affiliation

#### Conclusions

Ground water level was strongly associated with the presence of species in the community. Initial increase of ground water table degraded plant communities and enhanced the development of herbs, weeds and *Deschampsia caespitosa*. Further increase of soil moisture resulted in secondary habitat bogging. The communities of secondarily bogged habitats were mainly composed of species typical of bog sites and of occasionally noted endangered and protected species. High ground water level limited the growth and expansion of trees and

shrubs. High P content in soil enabled the development of syntaxa characteristic for the class *Phragmitetea*, like the association *Caricetum ripariae*.

After 30 years of drainage and agricultural activity on originally bogged sites, the bogging process returned. The secondarily bogged sites have good conditions for the return of ecologically valuable plant species and communities. They should be considered as ecological sites, because they protect water, soil, flora and fauna resources in natural environment.

#### References

Kozłowska T. and Frąckowiak H. (2011) Transformation of soil and meadow habitats as a result of changes in moisture and restricted management. *Polish Journal of Environmental Studies* 20(4A), 179–184.

Peeters A., Janssens F. and Decamps C. (1994) Importance of soil phosphorus on botanical diversity. *Proceedings of the 15<sup>th</sup> General Meeting of the EGF*, Wageningen, 231–232.

Sapek A. and Sapek B. (1997) Methods for chemical analysis of organic soils. Mat. Instruktażowe, 115, IMUZ, 80 (in Polish).

Traba Cz. (2001) Developmental trends of plant communities on meadows in the Łabuńka River valley. *Grassland Science in Poland* 4, 199–212 (in Polish with English summary).

# Seasonal aspects of marshy meadow communities

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# Abstract

Seasonally variable appearance of plant communities were evaluated in the course of fortnightly monitoring of the species composition of marshy communities. For this purpose, phytosociological reléves were taken every two weeks to estimate developmental phases of dominant plants, placing the main emphasis on the initiation of their flowering. On the same dates, biodiversity of the examined communities was assessed by calculating Shannon-Wiener index of the examined phytocenoses and determining site conditions of plant communities. It was concluded on the basis of the performed investigations that together with seasonal changes in the flora of the examined marshy meadow communities, also their natural values declined, despite increased biodiversity.

Keywords: seasonality, phytosociology, phenology, meadow communities, diversity

# Introduction

The species composition of plant communities and, consequently, of the biomass distribution, changes during the course of the vegetation season. This results, in part, from the different developmental rhythms of individual species, i.e. their phenological spectrum. Together with weather changes and simultaneous changes in site conditions, modifications in the floristic diversity of meadow communities take place, as well as in their natural values and sward fodder value score. Hence, this process exerts a significant impact on the appreciation of the plant community and can be utilized as an indicator of local phenological variability and also used to elaborate phonological-climatic maps of different regions (Podbielkowski and Podbielkowska, 1992; Sparks and Carey, 1995). The aim of the performed studies was to recognise seasonal floristic variability of marshy meadows and to assess, based on this background, changing biodiversity of these communities.

# Materials and methods

The investigations comprised the following representative areas of marshy meadow communities: *Phragmitetum australis*, *Caricetum gracilis* and community with *Deschampsia caespitosa*. Phytosociological reléves were achieved according to the Braun-Blanquet method, and phenological observations were performed in 2010 every two weeks from 9 April until the end of June on six permanent experimental plots for each community, measuring from 150 to 200 m<sup>2</sup>(108 phytosociological reléves). Observations of plant species in communities comprised changes in: numbers of species (in species composition), proportions in the soil cover as well as their developmental phase. Changes in the site conditions during the same period were measured on the basis of meteorological data (mean daily temperatures and total precipitations), and the phytoindication method (Ellenberg *et al.*, 1992) – taking into account values of the calculated indicators (F – humidity, R – soil reaction, N – nitrogen content in soil) which expressed the sum of products of the proportion of the species occurring at a given date and the value of the Ellenberg's index. Natural values of the meadow communities were assessed on the total number of species, the Shannon-Wiener index (Magurran, 2005) and the natural valorisation index (Oświt, 2000).

# Results

Together with successive observations in the course of the vegetative season, slight changes were found to occur in index values determining moisture content, soil reaction and soil nitrogen content. The examined sites were characterized by the highest moisture content at the beginning of the vegetation season, despite the fact that the beginning of 2010 was characterized by lower total precipitation than long-term means and, on the successive dates of measurements, their moisture levels depended on the sum of precipitation during the period of two weeks. The dependence of site moisture on precipitation was found to be higher for the community from the *Phragmitetea* class: *Phragmitetum australis* and *Caricetum gracilis*, in comparison with the *Deschampsia caespitosa* communities from *Molinio-Arrhenatheretea* class (Table 1).

Table 1. Weather conditions and values of selected site assessment indices of wet meadow communities: *Phragmitetum australis (Pa)*, *Caricetum gracilis (Cg)* and a community with *Deschampsia caespitosa (Dc)* 

Date	1.	0109.0	4.	10.0	42	6.04.	27.0	4.–1	0.05.	11.0	52	2.05.	23.0	)5.–7	.06.	8.06	521	.06.
Air temperature at the height of 2 m $-$ mean 24-hour (°C)																		
		0.3			9.0			12.5			11.8			15.1			17.7	
	Soil temperature at the level of 2 cm below surface – mean 24-hour (°C)																	
		15.3			8.8			10.8			10.5			12.3			14.4	
Sum of precipitation (mm)																		
		65.8			16.0	_		36.0			76.0			49.4			2.1	
			S	um o	f pre	cipit	atior	1 – m	lean	1971	-200	00						
		86.0			16.0			25.0			22.0			28.0			31.0	
	F	R	Ν	F	R	Ν	F	R	Ν	F	R	Ν	F	R	Ν	F	R	Ν
Pa	9.7	7.1	7.1	9.4	6.7	7.1	9.4	6.9	7.0	9.3	6.9	7.0	9.3	6.9	7.0	9.3	6.9	7.0
Cg	8.9	5.4	5.2	8.6	5.2	5.1	8.3	5.3	5.3	8.3	5.2	5.3	8.2	5.2	5.3	8.2	5.2	5.3
Dc	6.4	1.2	3.5	6.3	1.3	3.6	6.3	1.3	3.4	6.3	1.3	3.4	6.3	1.3	3.4	6.3	1.3	3.4

Ellenberg's index, F - moisture of soil, R - soil reaction, N - nitrogen content in soil.

The influence of atmospheric and site conditions was visible in changes in biodiversity of plant communities on consecutive dates of investigations (Table 2). The greatest number of plant species in the examined communities was recorded during the period with the highest sum of precipitation (9.04 and 22.05). On the other hand, numbers of flowering species present in the examined communities increased together with the increase of mean daily air temperature, especially after 22 May.

Table 2. Changes in natural	values of marshy	meadow phytocenos	ses during the	period of investigations

Plant community		]	Date of re	search in	2010 yea	r				
Plant community	09.04.	26.04.	10.05.	22.05.	7.06.	21.06.	Mean			
Number of species										
Phragmitetum australis	17	25	31	32	33	33	28.5			
Caricetum gracilis	17	21	28	35	42	42	30.8			
with Deschampsia caespitosa	18	25	30	32	33	33	28.5			
Number of flowering species										
Phragmitetum australis	0	5	8	10	18	25	_			
Caricetum gracilis	0	7	10	19	30	35	_			
with Deschampsia caespitosa	0	8	13	21	25	29	_			
Shan	non-Wiene	er floristic	diversity	index (H	')					
Phragmitetum australis	2,89	3.26	3.46	3.50	3.53	3.53	3.36			
Caricetum gracilis	2.89	3.13	3.40	3.61	3.61	3.78	3.43			
with Deschampsia caespitosa	3.35	3.65	3.82	3.89	3.92	3.90	3.76			
	Natur	al valoris	ation inde	ex						
Phragmitetum australis	2.94	2.92	2.81	2.76	2.71	2.71	2.80			
Caricetum gracilis	2.57	2.55	2.45	2.42	2.35	2.35	2.44			
with Deschampsia caespitosa	2.23	2.24	2.16	2.12	2.13	2.13	2.16			

Together with the advancement of the vegetation season, a slight value increase of Shannon-Wiener (H') index of floristic diversity was recorded in plant communities. On the other hand, natural values as determined by the Oświt index underwent a slight decrease as a result of incursion, together with the declining moisture content, of common species of wider ecological scale (Table 2). Together with changing site and weather conditions, also alterations in the phenological aspects of the examined phytocenoses associated with flowering of some plant species became apparent (Table 3).

26.04.	10.05.	22.05.	7.06.	21.06.			
Phragmitetum australis							
Phragmites australis	Phalaris arundi-	Dactylis glomerata	Rorippa amphibia	Potentilla anserina			
	nacea						
Carex riparia	Glechoma hederacea	Agrostis gigantea	Iris pseudoacorus	Scirpus sylvaticus			
C. gracilis			Achillea millefolium	Symphytum of-			
				ficinale			
C. acutiformis			Myosotis arvense				
Alopecurus pratensis							
Caltha palustris							
Caricetum gracilis							
Carex gracilis	Iris pseudoacorus	Agrostis gigantea	Alopecurus genicu-				
			latus				
Caltha palustris	Ranunculus repens	Cerastium vulgatum	Dactylis glomerata				
Equisetum palustre	Glechoma hederacea	Poa pratensis	Festuca arundinacea				
Rumex acetosa			Holcus lanatus				
Glechoma hederacea			Lolium perenne				
			Lychnis flos-cuculi				
			Phleum pratense				
			Scirpus sylvaticus				
			Trifolium pratense				
			Barbarea vulgaris				
com. with Deschampsia caespitosa							
Agrostis gigantea	Ranunculus repens	Rumex acetosa	Achillea millefolium				
Poa pratensis	Glechoma hederacea	Cardamine palustris	Ranunculus acris				
Holcus lanatus		Taraxacum of-	Glyceria maxima				
		ficinalis	-				
Cerastium vulgatum		Carex gracilis					
Phalaris arundinacea		-					

Table 3. Phenological phases of the evaluated communities of marshy meadows

#### Conclusions

Changing floristic composition of meadow grass communities characterised by the presence and domination of some flowering plant species illustrates their phenological aspects. Together with seasonal changes in the flora of the examined marshy meadow communities, also their natural values declined, despite increased biodiversity.

#### References

Ellenberg H., Weber H., Düll R., Wirth V., Werner W. and Paulissen D. (1992) Zeigerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotanica* 18, 1–258.

Magurran A.E. (2005) *Measuring biological diversity*. Blackwell Publishing, Malden: USA, 256 pp. Oświt J. (2000) *Metoda przyrodniczej waloryzacji mokradel i wyniki jej zastosowania na wybranych obiektach*. Wydawnictwo IMUZ. Materiały Informacyjne, 35 pp. (in Polish).

Podbielkowski Z. and Podbielkowska M. (1992) *Przystosowanie roślin do środowiska*. Warszawa, 570 pp. (in Polish).

Sparks T.H. and Carey P.D. (1995) The responses of species to climate over two century: an analysis of the Marsham phonological record. *Journal of Ecology* 83, 321–329.

# Species-rich *Nardus* grasslands – 'Natura 2000 habitats 6230' in the southern Brenta Dolomites (Trento, NE Italy)

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# Abstract

Species-rich *Nardus* grasslands have been indicated as 'Natura 2000 habitats 6230' by the Council Directive 92/43/CEE. Habitats 6230 have been described by several authors; however, the contributions of specific biodiversity, cover of *Nardus stricta*, and other acidophilic species to their coenoses are still unclear. Therefore, a study was established on three *Nardus* grassland areas of the southern Brenta Dolomites (Trento, NE Italy). During summer 2010, 94 plots were surveyed to compare their phytosociological characteristics to habitats 6230, and to investigate the contribution of specific biodiversity, *N. stricta*, and other acidophilic species to habitat 6230. Of the surveyed plots, 83% fitted the description of habitat 6230, and large variability occurred in terms of specific biodiversity, cover of *N. stricta*, number of other acidophilic species, and their cover.

Keywords: Nardus stricta, specific biodiversity, pasture, meadow, phytosociology

# Introduction

The definition of habitats 6230 (Council Directive 92/43/EEC) has been reported and interpreted by a number of authors for the European area and Italian environments. In the European literature, the habitats have been described as closed, dry or mesophile perennial *Nardus* grasslands which are remarkable for a high specific biodiversity (European Commission, 2003; 2007; Galvánek and Janák, 2008). In contrast, Italian manuals and reports empathized the dominance of *N. stricta* in these habitats (Lasen and Wilham, 2004; Lasen, 2006; Poldini *et al.*, 2006; Italian Ministry for Environment, 2010), or its prevalence in conjunction with other acidophilic species (Masutti and Battisti, 2008). A study was carried out to learn more about the botanical composition of habitats 6230 in meadows and pastures of the southern Brenta Dolomites (Trento, NE Italy). Other objectives were to determine the specific biodiversity, abundance of *N. stricta*, and presence of other acidophilic species; and to study the relationships occurring between these traits.

# Materials and methods

The work was carried out at three survey areas located in a Site of Community Importance (SCI IT3120009) of the southern Brenta Dolomites, characterized by the occurrence of habitats 6230 (Portale Geografico Trentino, 2011). In May 2010, experimental plots were established at 'Froschera Plain' (Area 1), 'Fontanelle' and 'Quadre' localities (Area 2), and the mountain agricultural farms 'Movlina' and 'Bregn de l'Ors' (Area 3). Meteorological parameters were collected from two weather stations located in proximity to the survey areas, having an annual precipitation  $\approx$  1,150 mm and an average temperature of 9.0°C (Pinzolo, 755 m a.s.l.), and 11.4°C (Stenico, 632 m a.s.l.). In the Area 1 (1,380–1,450 m a.s.l.) there were gently sloped meadows subjected to one harvest per year, mostly overrun by *Picea abies, Abies alba*, and *Fagus sylvatica*. Area 2 (1,750–1,850 m a.s.l.) consisted

of subalpine grasslands usually grazed by sheep and goats. Both Area 1 and Area 2 had substrates characterized by calcareous or dolomite rocks. Area 3 had two close pastures separated by a wood layer: one (1,750-1,900 m a.s.l.) was mostly plain with some *P. abies*, while the other (1,630-1,840 m a.s.l.) was on a SW-facing slope and was overrun by *P. abies* and *Larix decidua*. Substrate of Area 3 consisted of calcareous dolomites, or arenaria and granitoids. Plots of size 10 m by 10 m (Area 1 and Area 2, no. = 30; Area 3, no. = 34) were defined in order to have a cover of *N. stricta* ranging from 0% to maximum possible for each area. Botanical surveys were performed on three dates between May and September 2010 to evaluate the total number of species and their percent cover (Pirola, 1970). For each area, the specific biodiversity was subjected to multivariate analysis of variance using MULVA-5 (Wildi and Orlóci, 1996), and the clusters were subsequently processed to phytosociological analysis (Mucina *et al.*, 1993). Data of clusters not corresponding to habitats 6230 (Lasen, 2006) were excluded and SAS Proc Corr (ver. 9.2; SAS Institute, Cary, NC) was used to investigate the correlation between cover of *N. stricta* and the number of other acidophilic species, their cover, and specific biodiversity (total number of species).

#### **Results and discussion**

In the Area 1, 21 plots were identified as 6230 habitats as there were many species of the association *Gymnadenio-Nerdetum*. Among other plots, phytosociological analysis indicated that two constituted the association *Ranunculus bulbosi–Arrenatheretum*, while seven belonged to the *Bromion erecti* alliance. In Area 2, phytosociological analysis revealed 29 plots fitting to the description of habitat 6230, since there were species of the association *Homogyno* 



Figure 1. Correlation between cover of *Nardus stricta* and number of total species, number of other acidophilic species, and their cover in 'Natura 2000 habitats 6230' of three grassland areas in the southern Brenta Dolomites (Trento, NE Italy)

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*alpinae–Nardetum strictae* and *Sieversio montanae–Nardetum strictae*. The remaining plot was characterized by species belonging to the *Bromion erecti* alliance. In Area 3, six plots constituted the association *Crepido–Festucetum commutatae*, while the other 28 plots were identified as habitats 6230. These plots had a floral composition intermediate to the association *Homogyno alpinae–Nardetum strictae* and *Sieverso montanae–Nardetum strictae*; and sporadically contained species of *Crepido–Festucetum commutatae*. Among the plots fitting the description of habitat 6230 (83%), cover of *N. stricta* ranged between 1 and 80%, with an average of 34% (Figure 1). The total number of species ranged between 28 and 69, with an average of 47. Among these, the acidophilic species (except *N. stricta*) were 2 to 24 (average = 13), with cover values ranging between 2 and 43% (average = 15%). Significant but weak correlations were detected between cover of *N. stricta* and specific biodiversity or cover of other acidophilic species (Figure 1). Moreover, there was no correlation between cover of *N. stricta* and number of other acidophilic species.

## Conclusions

In three grassland areas of the southern Brenta Dolomites, the majority of coenoses were identified as habitats 6230. In these coenoses, large variability was found in terms of specific biodiversity, cover of *N. stricta*, number of other acidophilic species, and their cover. Thus, none of these traits had a neat threshold for identification of habitat 6230. Moreover, the increase of cover of *N. stricta* led to moderate decreases in specific biodiversity and cover of other acidophilic species.

## References

European Commission – DG Environment (2003) *The Interpretation Manual of European Union Habitats*, EUR 25, April 2003.

European Commission – DG Environment (2007) *The Interpretation Manual of European Union Habitats*, EUR 27, July 2007.

Galvánek D. and Janák M. (2008) Management of Natura 2000 habitats. 6230 \*Species-rich Nardus grasslands, European Commission.

Italian Ministry for Environment (2010) Italian interpretation manual of the 92/43/EEC Directive habitats. Lasen C. and Wilhalm T. (2004) Natura 2000 Habitat in Alto Adige, Autonomous Province of Bolzano-Alto Adige, Italy.

Lasen C. (2006) Habitat Natura 2000 in Trentino, Autonomous Province of Trento, Italy.

Masutti L. and Battisti A. (2007) La gestione forestale per la conservazione degli habitat della Rete Natura 2000, Veneto Region, Italian Academy of Forest Sciences, Venezia, Italy.

Mucina L., Grabherr G. and Ellmauer T. (1993) *Die Pflanzengesellschaften Österreichs*, vol. 3, Gustav Fischer, Jena, Germany.

Pirola A. (1970) Elementi di fitosociologia, Clueb, Bologna, Italy.

Poldini L., Oriolo G., Vidali M., Tomasella M., Stoch F. and Orel G. (2006) *Manuale degli habitat del Friuli Venezia Giulia. Strumento a supporto della valutazione d'impatto ambientale (VIA), ambientale strategica (VAS) e d'incidenza ecologica (VIEc)*, Autonomous Region of Friuli Venezia Giulia, Italy.

Portale Geografico Trentino (2011) *Cartografia habitat – Siti Natura 2000*. Online available: http://www.territorio.provincia.tn.it/portal/server.pt?open=514&objID=21153&mode=2

Wildi O. and Orlóci L. (1996) Numerical Exploration of Community Patterns. A guide to the use of MULVA-5, SPB Academic Publishing, Amsterdam, Netherlands.

# *Ex situ* evaluation of forage yield components and forage yields in wild populations of yellow vetchling (*Lathyrus aphaca* L.) from Serbia

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# Abstract

A small-plot trial was carried out in 2010 and 2011 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi, including six wild populations of yellow vetchling (*Lathyrus aphaca* L.) collected at diverse locations at the mountain Fruška Gora in Serbia. The proportion of leaf varied from 0.54 in the populations LA 05 and LA 06 to 0.61 in the population LA 01. The population LA 01 had the highest two-year average forage yields, namely 16.1 t ha<sup>-1</sup> of green forage and and 3.1 t ha<sup>-1</sup> of forage dry matter, while the population LA 06 had the lowest two-year average forage yields, namely 10.0 t ha<sup>-1</sup> of green forage and 2.0 t ha<sup>-1</sup> of forage dry matter. Satisfactory forage yields in certain wild populations and a short growing season offer solid grounds for the improvement and utilisation of yellow vetch to a greater extent.

Keywords: *ex situ* evaluation, forage yield components, forage dry matter yield, green forage yield, *Lathyrus aphaca*, yellow vetchling

# Introduction

Vetchlings (*Lathyrus* spp.) are one of the most numerous genera of the tribe *Fabeae* and the family *Fabaceae*, with around 160 species (Kenicer *et al.*, 2009). They are also widely represented in the wild and agricultural flora of the Balkan Peninsula, with about 30 species in present in Serbia. Although widely neglected and underutilised, grass pea (*L. sativus* L.) has the greatest economic importance as both food and feed (Vaz Patto, 2006; Mikić *et al.*, 2011). Little is known on the potential utilisation of other vetchlings as field crops, especially for forage production, although some references point out it could be great and economically justified (Bennett *et al.*, 1998). In order to acquire more knowledge on the subject, a long-term concerted action between the Institute of Field and Vegetable Crops and Faculty of Agriculture in Novi Sad targets collecting, preserving, maintaining, characterising and evaluating the wild populations and local landraces of diverse vetchling species in Serbia. The goal of the study was to evaluate forage yield components and forage yields in the wild populations of yellow vetchling (*Lathyrus aphaca* L.) of Serbian origin.

# Materials and methods

A small-plot trial was carried out in 2010 and 2011 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi. It included six wild populations of yellow vetchling collected at diverse locations at the mountain Fruška Gora in 2009. In both trial seasons all six populations were sown in early March, at a rate of 200 viable seeds m<sup>-2</sup> (Mihailović *et al.*, 2006–2007), a plot size of 5 m<sup>2</sup> and three replicates. Each population was cut in the stages of full flowering and first pods, as a balance between forage yield and quality, namely in the first half of May in both years. Plant height (cm), numbers of stems and lateral

branches (plant<sup>-1</sup>), number of internodes (plant<sup>-1</sup>), number of green leaves (plant<sup>-1</sup>) and leaf proportion were determined from the plant samples taken before cutting. Green forage yield (t ha<sup>-1</sup>) was measured *in situ* immediately after cutting. Forage dry matter yield (g plant<sup>-1</sup> and t ha<sup>-1</sup>) was determined upon the basis of a ratio between masses of green forage samples after and before the drying at room temperature. Results were processed by analysis of variance (ANOVA) applying the Least Significant Difference (LSD) test using the computer software MSTAT-C.

#### **Results and discussion**

On average, the effects were highly different the examined in forage yield components (Table 1). Populations differed significantly in plant height compared with the majority of the remaining tested wild populations: LA 01 had the greatest two-year average plant height (41 cm), while the populations LA 03 and LA 06 had the smallest average plant height (both 30 cm). The population LA 01 had the greatest average number of stems and lateral branches (10 plant<sup>-1</sup>), internodes (87 plant<sup>-1</sup>) and green leaves (78 plant<sup>-1</sup>). On the other hand, the population LA 06 had the smallest average number of stems and lateral branches (2 plant<sup>-1</sup>), internodes (25 plant<sup>-1</sup>) and green leaves (22 plant<sup>-1</sup>). The proportion of leaf in the total green forage yield per plant varied from 0.54 in the populations LA 05 and LA 06 to 0.61 in the population LA 01.

Unlike the effects on forage yield components, the effects related to forage yields (Table 2) were significant to a lesser extent. The average two-year green forage yield ranged between 10.0 t ha<sup>-1</sup> in the population LA 06 and 16.1 t ha<sup>-1</sup> in the population LA 01 (Table 2). With a similar trend, the variation of the average two-year forage dry matter yield ranged from 2.0 t ha<sup>-1</sup> in the population LA 06 to 3.1 t ha<sup>-1</sup> in the population LA 01. Generally, the forage yields in yellow vetchling, if grown as a forage field crop, are much lower than in forage pea, with an average of more than 30 t ha<sup>-1</sup> (Ćupina *et al.*, 2010a), or in grass pea, with an average of nearly 30 t ha<sup>-1</sup> (Mikić *et al.*, 2010), in the same agroecological conditions of the northern Balkans. According to its growth habit and its forage yields, it is anticipated that yellow vetchling could be intercropped in a rather similar way as bitter vetch (*Vicia ervilia* (L.) Willd.) and lentil (*Lens culinaris* Medik.) with short-stemmed field pea for forage production in temperate regions (Ćupina *et al.*, 2010b).

Population	Plant height (cm)	Number of stems and lateral branches (plant <sup>-1</sup> )	Number of inter- nodes (plant <sup>-1</sup> )	Number of green leaves (plant <sup>-1</sup> )	Leaf proportion
LA 01	41	10	87	78	0.61
LA 02	35	3	48	38	0.57
LA 03	30	3	45	41	0.57
LA 04	38	6	61	57	0.56
LA 05	32	3	36	27	0.54
LA 06	30	2	25	22	0.54
Mean	34	4	50	44	0.56
LSD <sub>0.05</sub>	6	4	26	22	0.04

Table 1. Average values of forage yield component in wild populations of yellow vetchling for the years of 2010 and 2011 at Rimski Šančevi

Dopulation	Green forage yield	Forage dry matter yield	
Population	$(t ha^{-1})$	$(t ha^{-1})$	
LA 01	16.1	3.1	
LA 02	11.4	2.3	
LA 03	11.6	2.2	
LA 04	14.2	2.7	
LA 05	10.6	2.0	
LA 06	10.0	2.0	
Mean	12.3	2.4	
LSD <sub>0.05</sub>	5.01	1.11	

Table 2. Average values of forage yield component in wild populations of yellow vetchling for the years of 2010 and 2011 at Rimski Šančevi

# Conclusions

Although these preliminary results show that yellow vetchling does not have as great a potential as a forage field crop as some other species of the same genus, there is a considerable variability in the agronomic characteristics related to forage in this species. Further collecting of its wild populations and their evaluation in field conditions will be continued. If taken into account together with its short growing season, in both natural habitat and field trials in Novi Sad, it offers solid grounds for the improvement and utilisation of yellow vetch to a greater extent.

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# References

Bennett S.J., Maxted N. and Sabanci C.O. (1998) The ecogeography and collection of grain, forage and pasture legumes in south-west Turkey. *Genetic Resources and Crop Evolution* 45, 253–262.

Cupina B., Krstić D., Antanasović S., Erić P., Pejić B., Mikić A. and Mihailović V. (2010a) Potential of the intercrops of normal-leafed and semi-leafless pea cultivars for forage production. *Pisum Genetics* 42, 11–14. Ćupina B., Mikić A., Stoddard F.L., Krstić D., Justes E., Bedoussac L., Fustec J. and Pejić B. (2011b) Mutual legume intercropping for forage production in temperate regions. In: Lichtfouse E. (ed.) *Sustainable Agriculture Reviews* 7: *Genetics, Biofuels and Local Farming Systems*, Springer, Dordrecht, pp. 347–365. Kenicer G., Nieto-Blásques E.M., Mikić A. and Smýkal P. (2009) *Lathyrus* – diversity and phylogeny in the genus. *Grain Legumes* 54, 16–18.

Mihailović V., Mikić A., Vasić M., Ćupina B. and Krstić D. (2006–2007) Possibilities for re-introduction of lentil (*Lens culinaris* Medik.) in Serbia. *Journal of Lentil Research* 3, 18–21.

Mikić A., Mihailović V., Ćupina B., Krstić Đ., Vasiljević S. and Milić D. (2010) Forage and seed yield components in four French landraces of grass pea (*Lathyrus* sativus L.). In: Huyghe C. (ed.) *Sustainable Use of Genetic Diversity in Forage and Turf Breeding*, Springer, Dordrecht, the Netherlands, pp. 127–130. Mikić A., Mihailović V., Ćupina B., Đurić B., Krstić D., Vasić M., Vasiljević S., Karagić Đ. and Đorđević V. (2011) Towards the re-introduction of grass pea (*Lathyrus* sativus) in the West Balkan Countries: the case of Serbia and Srpska (Bosnia and Herzegovina). *Food and Chemical Toxicology* 49, 650–654.

Vaz Patto M.C., Skiba B., Pang E.C.K., Ochatt S.J., Lambein F. and Rubiales D. (2006) Lathyrus improvement for resistance against biotic and abiotic stresses: From classical breeding to marker assisted selection. *Euphytica* 147, 133–147.
# The impact of grassland management on plant species diversity at a mesotrophic site in an eight-year-long experiment

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# Abstract

This paper is aimed at evaluating the botanical composition which changed under different grassland managements. At Jevíčko site in the Czech Republic, a long-term small-plot trial with tall oatgrass stand type (*Arrhenatherion*) was established on permanent grassland in 2003, consisting of 16 treatments in 4 replications. The intensity of utilisation was:  $I_1 = (1^{st} cut before 15 May, 4 cuts per year – cuts at 45-day intervals), <math>I_2 = (1^{st} cut between 16 and 31 May, 3 cuts per year at 60-day intervals), <math>I_3 = (1^{st} cut between 1 and 15 June, 2 cuts per year at 90-day intervals) and <math>I_4 = (1^{st} cut between 16 and 30 June, 1 or 2 cuts per year, second cut after 90 days). Four levels of fertilizer application were used: <math>F_0 = no$  fertilization, FPK =  $P_{30}K_{60}N_0$ ; FPKN<sub>90</sub> =  $P_{30}K_{60}N_{90}$ , FPKN<sub>180</sub> =  $P_{30}K_{60}N_{180}$ . In total, 87 plant species were identified between 2003 and 2011. Botanical composition was above all influenced by nitrogen fertilization, which supports grass species and reduces legumes and other forbs. Higher grass proportion was found in two-cut regimes ( $I_3$ ,  $I_4$ ), too. A higher diversity of plant species and a more balanced proportion of functional groups (grasses, legumes, forbs) were found especially in grassland without nitrogen fertilization.

Keywords: grasslands, cutting, fertilization, biodiversity, botanical composition

## Introduction

Agriculture in the Czech Republic (CR) has been considerably transformed after the reforms from the early 1990s, which has brought a livestock decrease by 50% and more, leading to deterioration of management and utilisation of permanent grasslands (Kohoutek *et al.*, 2009). The proportion of extensively utilised permanent grasslands has recently increased in the CR by up to 60–80% due to agroenvironmental measures, leading to a surplus of unfeedable forage (data from Ministry of Agriculture, Green report 2009). In Switzerland, the law requires that a minimum of 7% of total area consists of species-rich meadows and pastures (ecological compensation areas) with postponed first cut until 15 June in lowlands and 15 July in mountainous regions (Gujer, 2005). The goal is to reach about 10% of interconnected ecological compensation areas in Switzerland. For utilisation of every further 1% of the extensively managed permanent grasslands in the CR, it would be necessary to increase the livestock units (LU) numbers by 4000 heads which is not feasible presently, and so a 'vicious circle' arises (Kohoutek and Pozdíšek, 2006).

## Materials and methods

The long-term small-plot trial was performed on permanent grassland at the Jevíčko site between 2003 and 2011. The vegetation on the study site was classified as *Arrhenatherion*. The experimental site is located in a moderately warm and moderately wet region – B (Tolasz *et al.*, 2007) with altitude 343 m above sea level, annual average air temperature 7.4°C and annual average precipitation 545 mm (Tolasz *et al.*, 2007) given as the average for the years between 1966 and 1995. Soil type was classified as a fluvisol. In 2003 soil conditions were as follows: pH<sub>KCI</sub> 6.5, phosphorus (by Egner method) 37 mg kg<sup>-1</sup>, potassium (by Schachtschabel

method) 68 mg kg<sup>-1</sup> and magnesium 130 mg kg<sup>-1</sup>. Before experiment establishment, grassland had been utilized by a three-cut regime without fertilization over decades. There was a twofactorial design with four levels of each factor, cutting and fertilization, with all possible combinations, the total number of treatments being 16. The intensity of utilisation:  $I_1 = (1^{st})^{st}$ cut before 15 May, 4 cuts per year – cuts at 45-day intervals),  $I_2 = (1^{st} \text{ cut between } 16 \text{ and } 31)$ May, 3 cuts per year at 60-day intervals),  $I_3 = (1^{st} \text{ cut between } 1 \text{ and } 15 \text{ June, } 2 \text{ cuts per year at}$ 90-day intervals) and  $I_{4} = (1^{st} \text{ cut between 16 and 30 June, 1 or 2 cuts per year, second cut$ after 90 days). Four levels of fertilizer application:  $F_0 = no$  fertilization, FPK =  $P_{30}K_{60}N_0$ ;  $FPKN_{90} = P_{30}K_{60} + N_{90}$ ,  $FPKN_{180} = P_{30}K_{60} + N_{180}$ . Phosphorus was applied as superphosphate and potassium as potash salt, and nitrogen as calcium ammonium nitrate. The trial was set up in small plots with an area of 10 m<sup>2</sup> arranged as a randomized block design with 4 replications. Botanical composition of vegetation was assessed as projective dominance (cover) in every year and up to four times (because of up to four cuttings) per year. The botanical composition of vegetation was recorded as proportion of plant functional groups (grasses, legumes, forbs) and number of vascular plant species. The contribution evaluates the number of detected plant species per treatment (annual value contains all of the plant species detected up to four cuts per year and 10  $m^2$ ) and the effect of the treatments on the proportion of the plant functional groups averaged of over the years. The results were statistically analysed with a two-factor analysis of variance (ANOVA).

#### **Results and discussion**

The results of variance analysis (Table 1) show that intensity of utilisation and rate of fertilization highly significantly influence the proportion of grasses and legumes as well as the number of plant species in the grassland. The proportion of forbs is highly significantly influenced only by the rate of fertilization as well as legumes by interaction  $A \times B$ .

Table 1. Analysis of variance of evaluated features	(proportion of grasses,	legumes and	forbs in %
and plant species number as no. per 10 m <sup>2</sup> )			

		Evaluated feature							
Source of vari-	10 -	Grasses		Legumes Fc		Forb	s	Plant species number	
ability	di	SS	$F_{\text{test}}$	SS	$F_{\text{test}}$	SS	F <sub>test</sub>	SS	$F_{\text{test}}$
A (cuts)	3	1187	**	272	**	239	ns	1002	**
B (fertilization)	3	13268	**	748	**	7383	**	412	**
АхВ	9	419	ns	208	**	141	ns	30	ns
Total	63	20185		1550		11972		1697	

 $F_{\text{test}}$  – Fisher test; SS – sum of squares; df – degree of freedom; \*\* – statistically highly significant ( $P_{0.01}$ ); ns – statistically non-significant.

The effect of utilisation intensity and fertilization on the composition of functional groups and number of plant species is presented in Figure 1. For the average of years 2003 - 2011, grass proportion highly significantly increased from 4-cut utilisation ( $I_1 = 64\%$ ) towards two-cut utilisation ( $I_4 = 75\%$ ); also, progressive N fertilization increased grass proportion from 55% in the unfertilized control ( $F_0$ ) to 87% at the nitrogen rate of 180 kg ha<sup>-1</sup> (FPKN<sub>180</sub>). With increasing grasses proportion in the sward, the proportion of legumes and forbs decreased from 7% and 26%, respectively, in the 4-cut utilisation ( $I_1$ ) to 2% (highly significantly) and 22%, respectively, in the extensive two-cut utilisation ( $I_4$ ) (Figure 1). Increasing N fertilization also decreased the share of legumes and forbs highly significantly, from 6% and 37% ( $F_0$ ) to 0.3% and 11% (FPKN<sub>180</sub>), respectively.



Figure 1. The effect of utilisation intensity and fertilization level on the proportion of grasses, legumes, forbs and number of plant species in permanent grassland

Between 2003 and 2011 altogether 87 plant species were identified. In the extensive two-cut utilisation a lower number of species (18) was determined in comparison with the intensive 4-cut utilisation (28). The highest number of species was recorded on plots without (24) or with low (FPK) fertilization (24). A higher rate of N fertilization (180 kg  $ha^{-1}$ ) decreased the number of plant species down to 18.

Two-cut utilisation and increasing N fertilization promoted development and competitive ability of grasses, especially *Arrhenatherum elatius* and *Dactylis glomerata*, which reduced the proportion and number of other plants. These findings break the myth that an extensive two-cut utilisation of grasslands is a way towards higher diversity of grasslands. Recent research in the CR shows that the optimum extent of extensively managed permanent grasslands, cut in mid-June and utilised for cattle (dairy and beef cows) during interlactation period, should not exceed 15% of a managed area (Kohoutek and Pozdíšek, 2006).

## Conclusions

The attained results demonstrate that more frequent cutting systems (3-4 cuts) and extensive cattle management (decrease density LU per ha agricultural land by 50% and more) are optimum from an agricultural and ecological point of view, and also from the viewpoint of grassland diversity.

#### Acknowledgements

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## References

Gujer H.U. (2005) A policy to efficiently integrate biodiversity into grassland farming. *Grassland Science in Europe* 9, 73–79.

Kohoutek A. and Pozdíšek J. (2006) Fodder research in the Czech Republic focused on permanent grasslands (*in Czech*). In: Ferienčíková (ed.) *Grassland – part of mountain agriculture and landscape*. GMARI Banská Bystrica, pp. 21–27.

Kohoutek A., Kvapilík J., Cagaš B., Hrabě F. and Pozdíšek J. (2009) Selected indicators of productive and extraproductional management of grasslands in the Czech Republic. *Grassland Science in Europe* 14, 11–24. Tolasz R., Brázdil R., Bulíř O., Dobrovolný P., Dubrovský M., Hájková L., Halásová O., Hostýnek J., Janouch M., Kohut M., Krška K., Křivancová S., Květoň V., Lepka Z., Lipina P., Macková J., Metelka L., Míková T., Mrkvica Z., Možný M., Nekovář J., Němec L., Pokorný J., Reitschläger J.D., Richterová D., Rožnovský J., Řepka M., Semerádová D., Sosna V., Stříž M., Šercl P., Škáchová H., Štěpánek P., Štěpánková P., Trnka M., Valeriánová A., Valter J., Vaníček K., Vavruška F., Voženílek V., Vráblík T., Vysoudil M., Zahradníček J., Zusková I., Žák M. and Žalud Z. (2007) *Atlas podnebí Česka – Climate Atlas of Czechia*. 1<sup>st</sup> edition, ČHMÚ Praha, Olomouc, 255 pp.

# Impact of grassland management on occurrence of Arnica montana L.

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## Abstract

Due to changes in agriculture practice, the numbers of grassland species in Europe has declined within the last decades. Grassland management has, over the years, generated changes in floristic composition and determined the richness of plant diversity. On the natural grasslands of the Apuseni Mountains (Romania) the management also influences the presence or absence of important species like *Arnica montana* L. Habitats with *Arnica montana* L. are of great importance, both at a national and international level, due to the high conservative value of this species (listed in Natura 2000 Program, annex V, code number 6230). Meadows with *Arnica montana* L. have a high plant diversity both in terms of number of species and taxa of higher rank. Thus, their preservation is very important for the biodiversity of the region, where a subsistence agriculture is being practised.

Keywords: biodiversity, Apuseni Mountains, low input, Arnica montana

# Introduction

Low input farming systems can be defined as those which maximize the use of on-farm inputs. There are many different types of low input farming systems in Europe. They all are based on low use of external inputs such as agro-chemicals, artificial fertilizers, concentrate feedstuffs and water (irrigation). This generally goes together with lower outputs per hectare but also with higher non-commodity values, in terms of environment (water, air, soil and climate), biodiversity and/or landscape (Elbersen and Andersen, 2008). Extensive grazing and extensive meadow management practices have been typical for subsistence-based or small-scale farming systems in areas of low agriculture productivity. Our paper's objective is to describe the effect of traditional management on *Arnica montana* L. grassland as practised in the Apuseni Mountains, a mixed system of mowing and grazing (Rotar *et al.*, 2010). We also study how the management influences the biodiversity.

## Materials and methods

The experimental field is located in the Poienile Ursului, Garda de Sus village, Alba County, Apuseni Mountains at 1380 m elevation and started in 2009, using a randomized block design with five treatments with five replicates. The plot size is  $6 \text{ m}^2$  in the following experimental treatments: T1 – abandoned meadow, T2 – traditional mowing (once per year, at 5–7 cm cutting height, no later than 1 August), T3 – early mowing (once per year, at 5–7 cm cutting height, in the first part of June), T4 – mowing twice per year (at 5–7 cm cutting height, first time in June and the second in August), T5 – imitating grazing through mowing four times per year (in June, July, August and September, at 5 cm cutting height). The floristic studies have been performed according to Braun-Blanquet method. For floristic data analysis, we have used the PC-ORD Program which performs multivariate analysis of ecological data (McCune and Grace, 2002). Our emphasis is on nonparametric tools, graphical representation, randomization tests, and bootstrapped confidence intervals for analysis of community data and MRPP (Multi–response Permutation Procedures), which

is a nonparametric procedure for testing the hypothesis of no differences between two or more groups of entities. We have also used non-metric multidimensional scaling (NMS) which is an ordination method that is well suited to data that are non-normal or are on arbitrary, discontinuous, or otherwise questionable scales. NMS is generally the best ordination method for community data. A Monte Carlo test of significance is included.

#### **Results and discussion**

The application of the five types of management has lead to vegetation ordering in two groups of floristic composition, the first consisting of these types of management: abandonment, traditional mowing and mown twice per year, and the second group meets early mowing and grazing imitation (Figure 1). The plant community of the first treatment is not different in floristic composition from the plant community of the traditional mowing and the treatment which is mown twice per year (P > 0.05) but is different from early mowing and grazing imitation (P < 0.001; P < 0.01).



Figure 1. The floristic composition depending on the ordering of treatments (T-treatments, R-replication)

The floristic composition of the treatment that is traditionally mown does not differ from the plant community of the treatment mown twice per year (P > 0.05) but differs from the plant community of the treatment that is mown early and the treatment which imitates grazing (P > 0.01). The plant community of the treatment that is mown early and the treatment which imitates grazing (P > 0.01). The plant community of the treatment that is mown early is different in floristic composition from the treatment mown twice per year (P < 0.01) but is not different from the plant community treatment which imitates grazing (P > 0.05). The floristic composition of treatment which is mown twice per year is different from the grazing-imitation treatment (P < 0.001). Some species from canopy level occur only in the first group, e.g. *Hieracium aurantiacum*, L. *Polygala vulgaris* L., *Plantago lanceolata* L., *Trolius europaeus* L. etc., while others occur in the second group, such as: *Achillea distans* Waldst. et Kit., *Potentilla erecta* L., *Plantago lanceolata* L., Ranunculus bulbosus L., etc. Other species occupy an intermediate position, e.g. *Arnica montana* L. The presence of *Arnica montana* L. was

related to treatments that are traditionally mown or mown twice per year, but not to those mown early or where grazing was imitated. The plant community of the treatment of abandonment did not differ from the ones with treatments mown traditionally or twice per year, probably because the abandonment had started only in the year before the collection of data.

#### Conclusions

To preserve the botanical diversity of meadows in the Apuseni Mountains, and especially the presence of *Arnica montana* L., traditional management or mowing twice per year are recommended. However, longer term effects of these treatments need to be investigated.

#### Acknowledgements

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#### References

Elbersen B.S. and Andersen E. (2008) Low-input farming systems: Their general characteristics, identification and quantification. JRC (Joint Research Centre-European Comission), Italy, ISBN 978-92-79-08007-4, 12–21.

McCune B. and Grace J.B. (2002) Analysis of ecological communities. MJM Software Design, United States of America, ISBN 0-9721290-0-6, 188–198.

Rotar I., Pacurar F., Garda N. and Morea A. (2010) The management of oligotrophic grasslands and the approach of new improvement method. *Romanian Journal of Grassland and Forage Crops* 1, 57–70.

# Evolution of the botanical composition of meadows managed as set-aside strips to support wildlife

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# Abstract

Council Regulation (EC) 1782/2003, receipt by Italian DM (national law) 5406/2004 and DGR (regional law) 571/2005 of Veneto, has encouraged to set aside lands that were used for productive purposes. The DGR 571/2005 specified additional rules to increase the potentials of fauna associated with set-aside strips, especially within SPAs (Special Protection Area) and SCIs (Site of Community Importance). These rules recommended to make at least one cut per year and also to avoid harvesting from March to July, when mixed meadows are highly productive and normally harvested once or twice. A four-year study was conducted to investigate the effect of that management on three permanent mixed meadows in terms of botanical composition and soil coverage. Mixtures of grasses and legumes containing four, seven, and eight species were seeded in April 2005 at the Vallevecchia SPA/SCI (coastal plain of northeastern Italy), arranged in a completely randomized design with five replicates. The plots were managed according to the rules of DGR 571/2005, and their botanical compositions were assessed by surveys taken four times per year. The results pointed out that cespitose hemicryptophyte species (e.g. *Dactylis glomerata, Festuca arundinacea*) were more dominant and persistent compared to others, and weather patterns interacted with the meadows in determining soil cover.

Keywords: species dominance, mixed meadow, soil cover, legume, grass, linear transect

## Introduction

Over the last decades, the Common Agricultural Policy (CAP) has promoted to set aside lands that were cultivated by indicating several interventions, as receipt by Italian DM 5406/2004 and Venetian DGR 571/2005. In the Veneto Region, additional management strategies have been tailored by the DGR 571/2005 to protect and feed fauna, especially in SPAs and SCIs. While mixed meadows are usually subjected to harvests for forage purposes since spring re-growth until summer, these rules recommend the avoidance of harvesting from March to July. These recommendations are amended in order to not disturb breeding mammals and nesting birds; however, herbaceous plants are very competitive in this period, leading to high inter- and intra-specific selection. In addition, DGR 571/2005 indicates to harvest these set-aside areas at least once per year to prevent bushfires and maintain soil coverage, which is helpful to control weeds and to limit soil erosion. A four-year study was carried out in a SPA/SCI to assess the effects of such management strategies on botanical composition and soil cover of three mixed meadows. Results of this study can be useful to formulate meadow mixtures to be sown for similar purposes.

## Materials and methods

The experiment was carried out at the 'Vallevecchia' agricultural farm, in Caorle, northeastern Italy (45°37'33" N, 12°56'39" E; elevation  $\approx$ 0 m), which is sited along the Adriatic coast. The area has an annual average temperature of 12.6°C and an annual rainfall of 854 mm, distributed

following a bimodal pattern of precipitation. On 5 April 2005, three meadow mixtures were seeded in rows at a seeding rate of 25.8 kg ha<sup>-1</sup> on a silty clay loam soil. Mixtures included: (A) D. glomerata (20%), F. arundinacea (20%), Lolium perenne (20%), Festuca rubra (17%), Trifolium repens (9%), Medicago sativa (5%), Phleum pratense (5%), and Lotus corniculatus (4%); (B) F. arundinacea (18%), D. glomerata (17%), Lolium multiflorum (16%), L. perenne (16%), Trifolium pretense (16%), Ph. pratense (12%), and L. corniculatus (5%); (C) M. sativa (60%), D. glomerata (15%), L. perenne (15%), and T. pratense (10%). Plots (32 by 100 m) were arranged in a completely randomized design with five replicates. Harvests were taken two times per year, in late July and early September, from 2005 to 2008. Beginning in 2006, botanical composition and soil cover were surveyed in each May, July (before the first harvest), September (before the second harvest), and November (after the second harvest). The frequencies of mixture-species and weeds were determined using linear transects, by recording species in 20 cm segments of three lines of 5 m each (Daget and Poissonet, 1971). Soil cover ratings were based on a 0-100 scale, where 0 = bare soil, and 100 = complete plant coverage. For each meadow, specific frequencies were log-transformed and subjected to a repeated measure ANOVA to assess the effects of species, evaluation dates, and their interaction. Soil cover ratings were analysed to determine the effects of meadows, evaluation dates, and their interaction.

## Results

The ANOVA revealed that species × evaluation date and the main effects significantly affected (P < 0.0001) the species frequency of each meadow (Figure 1). Weed frequency decreased from  $\approx 10\%$  to  $\approx 3\%$  in each mixture throughout the study (data not shown); and *Ph. pratense* was not found in both mixtures A and B after the year of seeding. For each species, the frequency observed in May and June was higher than in September or November of each year (Figure 1), which can be explained by reduced plant size after harvesting. In mixture A, the frequency of *F. arundinacea*, *D. glomerata*, and *F. rubra* increased progressively from 2006 to 2008, whereas the frequency of *M. sativa* and *T. repens* markedly decreased. In mixture B, there was a consistent increase in *F. arundinacea* and *D. glomerata*, while the frequency of *T. pratense*, *L. multiflorum* and *L. perenne* decreased during the study period. In mixture C, a moderate increase in *D. glomerata* was observed from 2007 to 2008, counterbalanced by a slight decrease in the other three species. The interaction between mixture and dates, and the main effects were significant (P < 0.0001) for soil cover ratings (Figure 1). Mixture C displayed lower cover than mixture A in Nov. 2006; and lower than other two mixes meadows in Sept. 2007, Nov. 2007, Sept. 2008, and Nov. 2008 (Figure 1).

#### **Discussion and conclusions**

Managing meadows as set-aside strips to support wildlife can affect their botanical composition by advantaging large-size species and perennials, relative to small-size species (e.g. *T. repens*) and self-reseedings (e.g. *L. multiflorum*). Our findings are in agreement with several previous studies that reported a progressive reduction of specific biodiversity (e.g. number of species) under a limited number of cuttings (Thumm and Tonn, 2010; Gaisler *et al.*, 2011). Results of our study also suggest that species more effective in providing soil coverage are the cespitose hemicryptophytes (e.g. *D. glomerata*), especially those having short rhizomes (e.g. *F. arundinacea*, *F. rubra*). While differences among mixtures are of limited importance with regard to weed control, the mixed meadow with a high percentage of *M. sativa* provides the lowest soil cover for the winter period. This result suggests that sown meadow mixtures with a high percent of *M. sativa* may lead to high inter- and intra-specific competition in set-aside strips.



Figure 1. Specific frequency and soil cover of three meadows (Mixtures A–C) as affected by evaluation date from May 2006 to Nov. 2008 in Caorle, NE Italy. Data points represent the average of five replicates; error bars indicate  $\pm$  SE. Different letters denote statistical differences according to Tukey's HSD test ( $\alpha = 0.05$ )

#### References

Daget P. and Poissonet T. (1971) Une méthode d'analyse phytologique des prairies. *Annales Agronomiques* 22, 5–41.

Gaisler J., Pavlů V. and Pavlů L. (2011) Effect of different extensive management treatments on the plant diversity of an upland meadow without forage utilization. *Grassland Science in Europe* 16, 577–579. Thumm U. and Tonn B. (2010) Effect of precipitation on dry matter production of a meadow with varied

Thumm U. and Tonn B. (2010) Effect of precipitation on dry matter production of a meadow with varied cutting frequency. *Grassland Science in Europe* 15, 90–92.

# Functional diversity-area relationship in permanent grassland

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# Abstract

The target of a multi-functional, sustainable use of grasslands requires deeper understanding of the relationships between grassland management and plant diversity and its consequences on ecosystem functioning. Some of these relationships are well known for specific pedoclimatic conditions. One option to extend our knowledge to a broader range of conditions is to aggregate different studies by performing a meta-analysis. Nevertheless, differences in sampling area between studies are a major challenge. For taxonomic diversity, species-area curves have been established for different habitats but references for functional diversity are scarce. We aimed at assessing the possibility of using functional diversity-area curves to correct for differing sampling area between studies before merging data for overall analysis on functional diversity. We measured diversity in 9 nested areas of increasing size (from 0.01 m<sup>2</sup> to 100 m<sup>2</sup>) in 16 grasslands with different management (grazing, mowing).We analysed the effect of area on specific richness, functional traits (SLA, LDMC) and on the percentage of legumes. We conclude that correction for differing sampling area is possible for functional richness and community-weighted mean values.

Keywords: plant functional traits, functional diversity, biodiversity-area relationships, survey minimal area

## Introduction

One argument for the preservation of permanent grassland in ruminant-based farming systems is the provision of several ecosystem services. Ecosystem services (ES) are the services provided by the ecosystem for mankind. ES can be linked to some functional diversity criterion (de Bello *et al.*, 2010). Functional diversity (FD) is the set of values of a functional trait (or multiple traits) of the individuals (or species) of a community. Functional diversity is driven by the environmental conditions (agricultural practices and pedoclimatic conditions). The relationships between FD and ES and between FD and environmental factors are generally studied only for local conditions. The generalization of these relationships has to be made at larger scales. One solution is to gather the different local studies. The main problem is the differences of survey protocol between studies, especially in terms of the sampling area of the survey. Our goal is to assess the relationships between sampling area between studies.

## Materials and methods

We studied 16 permanent grasslands with contrasting management conditions in Northeastern France in 2010. The botanical composition was recorded on 9 different nested square areas ( $0.01 \text{ m}^2$ ,  $0.0625 \text{ m}^2$ ,  $0.25 \text{ m}^2$ ,  $0.5625 \text{ m}^2$ ,  $1 \text{ m}^2$ ,  $6.25 \text{ m}^2$ ,  $25 \text{ m}^2$ ,  $56.25 \text{ m}^2$ ,  $100 \text{ m}^2$ ). For quadrats of less than  $1 \text{ m}^2$ , the abundances of species were visually estimated over the whole surface. For surfaces greater than 1 m<sup>2</sup>, the abundances were visually estimated by subsampling several quadrats of  $0.25 \text{ m}^2$ . The number of quadrats was proportional to the surface (2 for 6.25 m<sup>2</sup>, 4 for 25 m<sup>2</sup>, 7 for 56.25 m<sup>2</sup>, and 11 for the 100 m<sup>2</sup>). Different plant diversity criteria were considered: the total number of species, the percentage of legumes in the sward, the aggregated trait of Leaf Dry Matter Content (LDMC) and Specific Leaf Area (SLA). The aggregated trait is the sum of the trait of each species weighted by its relative abundance. It represented the average trait of the community. We also studied the functional amplitude of LDMC and SLA, as the difference between the minimum trait value and the maximum trait value of the community, and the Rao index (Botta-Dukat, 2005) of these two traits. The values of the functional traits per species were taken from the LEDA trait database (Klever et al., 2008). These criteria were calculated using the FD package on R 2.13.1 (Laliberté and Legendre, 2010). The links between biodiversity criterion and the sampling area was studied using the model Criterion =  $a \times log (area) + b$ . We calculated the area required to detect  $\pm$  5% of the diversity (minimal area) by linear interpolation between the measures. If the value of the diversity criteria was increasing with the sampling area, the minimal area was considered as the smallest area with 95% of the biodiversity of the 100 m<sup>2</sup> area. For diversity criteria values decreasing with the sampling area, the area with 105% of the biodiversity of the 100 m<sup>2</sup> area was considered minimal.

#### Results

We found a close relationship between the sampling area and the number of species (Figure 1a), the functional amplitude of LDMC (Figure 1b) and of SLA (Table 1). For the other diversity criteria, no relationships with the area were found. The two aggregated traits have the smaller minimal area among all indices (around 2 m<sup>2</sup>). The other indices have a minimal area bigger than 10 m<sup>2</sup> (Table 1).

6			
Diversity criterion DC	Minimal area (m <sup>2</sup> )	Overall Model	$R^2$
Number of species	59.16	$1.49 \times \log(\text{Area}) + 15.35$	0.35*
% legumes	54.90	$-1.0 \times \log(Area) + 21.99$	0.05 ns
Aggregated Trait of LDMC	1.61	$0.23 \times \log(Area) + 214.87$	0.003 ns
Aggregated Trait of SLA	2.09	$-0.10 \times \log(\text{Area}) + 26.59$	0.02 ns
FALDMC	26.20	$0.028 \times \log(\text{Area}) + 0.679$	0.23*
FA SLA	14.25	$0.015 \times \log(Area) + 0.694$	0.11*
Rao LDMC	31.45	$0.00 \times \log(Area) + 0.481$	0.00 ns
Rao SLA	55.72	$0.015 \times \log(Area) + 0.694$	0.00 ns

Table 1. Relationships between area and biodiversity (AT: aggregated trait, FA: functional amplitude; ns *P*-value not significant; \* *P*-value significant)

## Conclusions

Functional amplitude and the number of species can be related to the sampling area. A model of these relationships can be used to match data coming from various origins, differing in the sampling areas. For the aggregated traits, the minimal area is very small. All studies with a sampling area greater than  $2 \text{ m}^2$  can be aggregated without correction. For the other indices (Rao and % of legumes), the minimal area is large and no relationship with the sampling area was found. Thus harmonization for these indices seems impossible. This preliminary work shows some ways to deal with difference in survey protocol for meta-analysis. However, further studies with a larger number of grasslands in a wider range of conditions should be performed to ascertain these conclusions.



Figure 1. Evolution of selected biodiversity criteria with sampling area for 5 grasslands. The vertical line represents the minimal area and the bold curve represents the overall model: a) relationship with the number of species; b) With the Leaf Dry Matter Content (LDMC) amplitude; c) with the Specific Leaf Area (SLA) Aggregated trait; and d) the percentage of legumes

#### References

Botta-Dukat Z. (2005) Rao's quadratic entropy as a measure of functional diversity based on multiple traits. *Journal of Vegetation Science* 16, 533–540.

de Bello F., Lavorel S., Diaz S., Harrington R., Cornelissen J.H.C., Bardgett R.D., Berg M.P., Cipriotti P., Feld C.K., Hering D., da Silva P.M., Potts S.G., Sandin L., Sousa J.P., Storkey J., Wardle D.A. and Harrison P.A. (2010) Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity and Conservation* 19, 2873–2893.

Kleyer M., Bekker R.M., Knevel I.C., Bakker J.P., Thompson K., Sonnenschein M., Poschlod P., Van Groenendael J.M., Klimes L., Klimesová J., Klotz S., Rusch G.M., Hermy M., Adriaens D., Boedeltje G., Bossuyt B., Dannemann A., Endels P., Götzenberger L., Hodgson J.G., Jackel A.K., Kühn I., Kunzmann D., Ozinga W.A., Römermann C., Stadler M., Schlegelmilch J., Steendam H.J., Tackenberg O., Wilmann B., Cornelissen J.H.C., Eriksson O., Garnier E. and Peco B. (2008) The LEDA Traitbase: A database of life-history traits of the Northwest European flora. *Journal of Ecology* 96, 1266–1274.

Laliberté E. and Legendre P. (2010) A distance-based framework for measuring functional diversity from multiple traits. *Ecology* 91, 299–305.

# Occurrence of *Trifolium repens* L. in communities of *Molinio-Arrhenatheretea* in the mountain-foot regions of SE Poland

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# Abstract

Based on 900 phytosociological releves representing mountain-foot extensive meadows and pastures, 12 plant communities were distinguished. For the releves with *Trifolium repens* the average values of ecological indices L, F, R and N were calculated and analysed. Soil samples were analysed with respect to pH, phosphorus, potassium and magnesium. *Trifolium repens* occurred more frequently and more abundantly in the communities of *Arrhenatheretalia* than of *Molinietalia* order, except for the community with *Holcus lanatus*. A substantial share of *T. repens* in swards of mountain-foot pastures and periodically grazed meadows is important because of their extensive use and limited fertilization. In the same time the results of phyto-indicative analyses point at a broad spectrum of *T. repens* ecological preferences, including good insolation, moderate moisture, slightly acidic or neutral soils moderately rich in nitrogen.

Keywords: *Trifolium repens*, frequency, communities, *Molinio-Arrhenatheretea*, soil properties, phyto-indication

## Introduction

*Trifolium repens* is the most important papilionaceous pasture plant in Europe. This is because of its persistency, broad ecological spectrum, and considerable resistance to the intensity of use, as well as for economic and environmental reasons (Novoselova and Frame, 1992). A contribution of 40% of *T. repens* in a meadow sward can substitute for 120 kg N ha<sup>-1</sup> of mineral nitrogen (Hayness *et al.*, 1970). However, in order to secure the persistence of *T. repens*, its share should not exceed that figure. The goal of this study was to define the abundance of *T. repens* in plant communities of *Molinio-Arrhenatheretea* class with regard to selected ecological factors.

## Materials and methods

The phytosociological studies based on the Braun-Blanquet method were carried out on extensively used and sporadically fertilized meadows and pastures of the Przemyskie Foothills and Dynowskie Foothills (Western Carpathians with Podkarpacie province). The area is dominated by brown soils, luvisols and alluvial soils developed from dusts and loams. The length of the growing season ranges from 210 to 225 days, average annual precipitation 700–800 mm, and average annual temperature is 7.3°C. On the basis of 900 releves, made on 100 m<sup>2</sup> plots in 1999–2009, plant associations and communities were distinguished. The frequency, cover coefficient, and distribution of *T. repens* abundance degrees were determined. Based on the floristic composition of all releves with *T. repens* (in total 614), average values of L, F, R and N indices were calculated by the method of Ellenberg *et al.* (1992). The results were tested statistically with Statistica v. 9 (StatSoft Inc. 1984–2010). Soil pH (in KCl) and available forms of P, K and Mg were analysed in soil samples collected from the 5–15 cm layer.

#### **Results and discussion**

*Trifolium repens* occurred in twelve associations and communities of the *Molinio-Arrhenatheretea* class, including five assigned to *Molinietalia*, six assigned to *Arrhenatheretalia*, and one assigned to *Plantaginetalia majoris* orders. Similarly to the San valley (Traba *et al.*, 2005), the optimal conditions for *T. repens* occurred in short pasture sward of *Lolio-Cynosuretum* and in heavily trampled *Lolio-Polygonetum*, where the species often reached the highest abundance degrees. It was also frequently and abundantly found on periodically grazed meadows of the *Trisetetum flavescentis* association, as well as in *Poa pratensis-Festuca rubra* and *Holcus lanatus* communities. The smallest cover coefficient for *T. repens* was found in tall sward of the *Cirsietum rivularis*, where it occurred at low levels of abundance (Table 1). In the same associations, but on organic soils, *T. repens* occurred with lower frequency and cover (Wyłupek and Trąba, 2003). The soils under associations and communities with *T. repens* usually had acidic reaction, very low quantity of phosphorus, moderate quantity of potassium and high magnesium (Table 2).

Table 1. Frequency and quantitative relations of Trifolium repens in the distinguished communities

Association, com-	Releve no	Frequency Cover coef-		Distribution of levels of abundance %				
munity	Refeve no.	%	ficient	+	1	2	3	4
Ss	47	53.2	374	28.0	52.0	16.0	4.0	0.0
Cr	41	31.7	173	53.8	23.1	23.1	0.0	0.0
Dc	42	30.9	198	46.1	38.5	7.7	7.7	0.0
Hl	123	74.8	757	29.3	34.8	26.1	9.8	0.0
Ap	74	47.3	368	42.9	28.6	22.8	5.7	0.0
Âe	194	64.4	572	40.8	28.8	20.8	9.6	0.0
Tf	49	100.0	1606	18.4	30.6	28.6	18.4	4.0
<i>PpFr</i>	67	98.5	1042	16.7	42.4	34.8	6.1	0.0
Fr	49	55.1	198	51.9	40.7	7.4	0.0	0.0
Ac	67	59.7	486	40.0	37.5	15.0	5.0	2.5
LC	98	95.9	2619	5.3	11.7	39.4	29.8	13.8
Lpa	49	88.9	1766	2.8	194	30.6	47.2	0.0

Table 2. Soil characteristics

Association, com-	Number of samples	nH in KCl	Р	K	Mg
munity	Number of samples	pii iii KCi -		mg kg <sup>-1</sup>	
Ss	7	5.9	21.2	88.3	163.4
Cr	5	6.0	15.1	76.5	181.5
Dc	6	4.0	14.6	118.0	124.6
Hl	28	4.8	16.1	93.5	150.8
Ap	10	4.8	14.2	92.6	133.3
Âe	22	4.7	17.4	123.5	144.3
Tf	13	5.1	11.3	111.0	216.2
<i>PpFr</i>	15	4.9	17.3	123.7	97.7
Fr	8	4.9	12.3	77.9	140.7
Ac	10	4.0	14.4	101.1	111.4
LC	13	4.7	26.8	107.3	129.3
Lpa	10	3.9	18.9	121.5	81.4

Explanations: Molinietalia: Ss-Scirpetum sylvatici, Cr-Cirsietum rivularis, Dc-with Deschampsia caespitosa, Hl-with Holcus lanatus, Ap-Alopecuretum pratensis; Arrhenatheretalia: Ae-Arrhenatheretum elatioris, Tf- Trisetetum flavescentis, PpFr-Poa pratensis-Festuca rubra, Fr-with Festuca rubra, Ac-with Agrostis capillaris, LC-Lolio-Cynosuretum; Plantaginetalia: LPa-Lolio-Polygonetum

In 27% of the studied sample plots *T. repens* occurred with '+', in 30% - 1, 26% - 2, 17% - 3 and 4 abundance degrees of the Braun-Blanquet scale. The range of analysed indices L, F, R, N was relatively broad (Figure 1). However, according to the L, F, R and N ecological indices, calculated on the basis of the floristic composition of phytocenoses with higher *T. repens*, abundancies (2–4), showed that the species prefers sites of good insolation, soils with moderate moisture, slightly acidic or neutral pH, and moderately rich nitrogen content. The *T. repens* tolerance to various soil conditions (mineral and organic



Figure 1. Comparison of the L (solar radiation), F (soil moisture), R (soil reaction) and N (soil nitrogen content) ecological number for *Trifolium repens* with respect to its abundance

soils), moisture (communities on wet, humid, mesic, dry and poor sites) was reported by Grynia *et al.* (1997), Wyłupek and Trąba (2003) and Trąba *et al.* (2005).

#### Conclusions

*Trifolium repens* occurred more frequently and more abundantly in the communities of the *Arrhenatheretalia* class than of the *Molinietalia* class, except for the *Holcus lanatus* community. A substantial share of *T. repens* in the sward of mountain-foot pastures and periodically grazed meadows is important because of their extensive use and limited fertilizer supply, in particular nitrogen. The results of phyto-indication analyses revealed both a broad ecological spectrum of *T. repens* and its preferences to some key environmental conditions.

#### References

Ellenberg H., Weber H.E., Düll R., Wirth V., Werner W. and Paulissen D. (1992) Zeigerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotica* 18, 258 pp.

Grynia M., Kryszak A. and Grzelak M. (1997) Ecological conditions of the occurrence of legumes in meadowpasture communities in Great Poland. *Grassland Science in Europe* 2, 301–305.

Hayness D., Hunt J.V. and Frame J. (1970) Yield, root morphology and chemical composition of two legumes as affected by lime and phosphorus application to an acid soil. *Plant and Soil* 62, 2, 241–254.

Novoselova A. and Frame J. (1992) The role of legumes in European grassland production. *Proceedings of the 14<sup>th</sup> General Meeting of the European Grassland Federation*, Lahti, pp. 87–96.

Traba Cz., Wolański P. and Oklejewicz K. (2005) Share of legumes in selected non-forest plant communities in the San valley. *Grassland Science in Europe* 10, 173–177.

Wyłupek T. and Trąba Cz. (2003) The occurrence of legumes in meadow communities of the Por River valley. *Grassland Science in Europe* 8, 602–605.

# The floristic composition of extensive pastures in the valley of the Wieprz river

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# Abstract

The objective of this study was to assess the floristic composition of extensive pastures for dairy cattle grazing. The pastures are located in the valley of the Wieprz river in Tarnogóra (Izbica commune, Lublin Province), and belong to a "Land Community" established more than 100 years ago. Currently the Land Community manages about 200 hectares of land, including 132 ha of pastures, 55 ha of forests and 13 ha of arable land. Cow grazing begins around 1 May and lasts approximately until 1 November. Within the phytocenoses distinguished, the dominant species were *Lolium perenne*, *Deschampsia caespitosa*, *Agrostis stolonifera*, *Cynosurus cristatus*, *Poa pratensis*, *P. trivialis*, *Agrostis capillaris* and *Festuca pratensis* among grasses; *Trifolium repens* and *T. pratense* among legumes, and *Ranunculus repens* and *Taraxacum officinale* among other botanical families. Ellenberg indicator values were used to assess the habitat conditions, i.e. soil reaction (R), moisture (F) and nitrogen content (N).

Keywords: plant associations and communities, extensive pastures

## Introduction

The floristic composition of pastures depends on many factors: habitat conditions (particularly soil humidity and fertility), species and stocking rate of animals (Rogalski *et al.*, 1999; Warda and Rogalski, 2004), as well as the system of grazing and maintenance used. The selective intake of species and leaving of droppings by animals also impacts on the species composition of the sward (Rogalski *et al.*, 2000).

The study objective was to assess the floristic composition of extensive pastures for dairy cattle grazing.

## Materials and methods

The investigation was conducted on extensive pastures located in the valley of the Wieprz river in Tarnogóra (Izbica commune, Lublin Province). This part of the Wieprz valley belongs to a large Natura 2000 habitat area PLH060030 – the Izbica Wieprz Gap. The pastures are located on muck and bog soils originating from mineral alluvial and diluvial sediments, rich in organic matter, and are characterised by varying water content (from the optimum to boggy to periodically waterlogged and flooded). The pastures belong to the so-called Land Community, established more than 100 years ago. Currently the Land Community manages about 200 hectares of land, including 132 ha of pastures, 55 ha of forests and 13 ha of arable land. Cow grazing begins around 1 May and lasts approximately until 1 November. In the spring of 2011, a total of 38 phytosociological relevés according to Braun-Blanquet were made on the pastures. Ellenberg indicator values were used to assess the habitat conditions in the associations investigated in this study, i.e. soil moisture (F), soil reaction (R), and nitrogen content (N) (Ellenberg *et al.*, 1992). The species names were given according to Mirek *et al.* (2002).

## **Results and discussion**

The study presents the floristic composition of 4 selected plant associations and 1 community belonging, according to Matuszkiewicz (2006), to the class *Phragmitetea* R. Tx. et Prsg 1942 (*Glycerietum maximae* Hueck 1931 and *Caricetum acutiformis* Sauer 1937) and *Molinio-Arrhenatheretea* R. Tx. 1937 (*Scirpetum silvatici* Ralski 1931, community *Deschampsia caespitosa* and *Lolio-Cynosuretum* R. Tx. 1937).

The *Lolio-Cynosuretum* association and *Deschampsia caespitosa* community occupied 70%, and the *Scirpetum silvatici*, *Glycerietum maximae* and *Caricetum acutiformis* communities occupied 30% of the pasture area (Table 1). A total of 36 plant species occurred on the pastures, although up to 124 plant species may occur in pasture communities in the river valleys of the Lublin Region (Mosek, 2000).

Plant associations	Lolio-Cyno-	Communities	Scirpetum	Glycerietum	Caricetum
and communities	suretum T.Tx.	Deschampsia	silvatici Ral-	maximae	acutiformis
and communities	1937	caespitosa	ski 1931	Hueck 1931	Sauer 1937
Area occupied (%)	25	45	10	10	10
Number of phytosocio-	10	10	6	6	6
logical relevés	10	10	0	0	0
Total number of species	21	25	12	14	13
Number of species (range	15 21	10.25	0.12	0.14	13 15
from-to)	15-21	19-23	9-12	9-14	15-15
Mean species num-	10.1	22.4	10.7	12.5	1/1 3
ber per releve	19.1	22.4	10.7	12.5	14.5
Share group of plants (%)					
Grasses	47.6	44.0	25.0	28.6	30.8
Legumes	9.5	12.0	8.4	7.1	0.0
Sedge and juncus	9.5	8.0	33.3	14.3	7.7
Herbs and weeds	33.4	36.0	33.3	50.0	61.5
Moisture (F)	5.72	5.83	7.87	8.44	8.02
Soil reaction (R)	2.25	1.67	2.60	4.78	3.99
Soil nitrogen content (N)	5.40	4.54	4.83	6.92	5.24

Table 1. Description of species diversity, soil moisture (F), soil reaction (R), and nitrogen content (N) of the analysed plant associations and communities

The largest number of species was found in the Deschampsia caespitosa community (25 species) and the Lolio-Cynosuretum association (21 species). These associations were characterised by a large share of grasses (47.6 and 44.0%) and plants classified as herbs and weeds (33.4 and 36.0%), and a smaller share of plants in the family Fabaceae (9.5 and 12.0%), Cyperaceae and Juncaceae (9.5 and 8.0%). The average number of species was 19.1 (15–21) for the Lolio-Cynosuretum association, and 22.4 (19–25) for the Deschampsia caespitosa community. Cynosurus cristatus L. (3-4), Lolium perenne L. (3-4), Trifolium repens L. (3-4) were characteristic species of the *Lolio-Cynosuretum* association, whereas Deschampsia caespitosa (L.) P. Beauv. (3-4) was characteristic of the Deschampsia caespitosa community. Besides the species mentioned above, the Lolio-Cynosuretum association and the Deschampsia caespitosa community also featured two species belonging to the Cynosurion R.Tx.: Leontodon autumnalis L. (+-1) and Bellis perennis L. (+-1) alliance. The Molinio-Arrhenatheretea class was represented by a greater number of species: Poa pratensis L., Festuca pratensis L., Festuca rubra L., Plantago lanceolata L., Poa trivialis L., Trifolium pratense L., Phleum pratense L., Galium mollugo L. and Prunella vulgaris L. Species belonging to the Agropyro-Rumicion crispi Nordh. 1940 em. R.Tx. 1950 alliance were also found to occur in the *Lolio-Cynosuretum* association and the *Deschampsia caespitosa* community, and these species included: *Agrostis stolonifera* L., *Ranunculus repens* L., *Potentilla anserina* L., *Carex hirta* L. and *Elymus repens* (L.) Gould. The occurrence of these species is a result of extensive pasture management (Kryszak, 2004).

Herb and weed species (33.3–61.5%) and species in the *Poaceae* family (25.0–30.8%) dominated in the phytocoenoses *Scirpetum silvatici*, *Glycerietum maximae* and *Caricetum acutiformis*. The characteristic species in these three plant associations were, respectively, *Scirpus sylvaticus* L. (sward coverage rate 4), *Glyceria maxima* (Hartm.) Holmb. (3–4) and *Carex acutiformis* L. (3–4). In addition, a considerable share in these phytocoenoses was observed for the following species in the *Phragmitetea* class: *Galium palustre* L., *Poa palustris* L., *Caltha palustris* L. and *Rorippa amphibia* (L.) Besser, as well as species in the *Agropyron-Rumicion crispi* alliance: *Agrostis stolonifera* L., *Ranunculus repens* L., *Lysimachia numnularia* L., *Potentilla anserina* L., *Carex hirta* L. and *Rumex crispus* L. In all plant associations, the accompanying species had a small percentage share, which indicates their accidental presence in a given community.

The *Lolio-Cynosuretum* association and *Deschampsia caespitosa* community occurred in habitats with similar moisture rates (F = 5.72-5.83), soil reaction (R = 1.67-2.25), and nitrogen content (N = 4.54-5.40). The *Scirpetum silvatici*, *Glycerietum maximae* and *Carice-tum acutiformis* associations occurred in habitats with higher moisture rates (F = 7.87-8.44), higher value of soil reaction (R = 2.60-4.78), and higher nitrogen content (N = 4.83-6.92).

#### Conclusions

On the extensive pastures in Tarnogóra, the *Lolio-Cynosuretum* association and *Deschampsia caespitosa* community characterised by a large species diversity were dominant and occurred in moderately humid habitats. The *Scirpetum silvatici*, *Glycerietum maximae* and *Caricetum acutiformis* associations occupied a smaller area, demonstrated a smaller floristic diversity, and occurred in habitats excessively humid periodically.

In the dominant associations, the dominant species were *Lolium perenne*, *Deschampsia caespitosa*, *Agrostis stolonifera*, *Cynosurus cristatus*, *Poa pratensis*, *Poa trivialis*, *Agrostis capillaris* and *Festuca pratensis* among grasses, *Trifolium repens* and *T. pratense* among legumes, and *Ranunculus repens* and *Taraxacum officinale* among other botanical families.

## References

Ellenberg H., Weber H.E., Düll R., Wirth V., Werner W. and Paulissen D. (1992) Zeigerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotanica* 18, 258 pp.

Matuszkiewicz W. (2006) Przewodnik do oznaczania zbiorowisk roślinnych. PWN, Warszawa, 537 pp.

Mirek Z., Piękoś-Mirkowa H., Zając A. and Zając M. (2002) Flowering plants and pteridophytes of Poland. A checklist. Kraków. W. Szafer Institute of Botany PAS, 442 pp.

Mosek B. (2000) Wpływ składu florystycznego zbiorowisk pastwiskowych dolin rzecznych Lubelszczyzny na ich wartość paszową. *Zeszyty Naukowe AR w Krakowie* 368, 235–240.

Kryszak A. (2004) Synantropizacja wybranych zbiorowisk łąkowych. *Woda-Środowisko-Obszary Wiejskie* 4, 1(10), 201–208.

Rogalski M., Wieczorek A. and Kardyńska S. (1999) Pasące się zwierzęta jako czynnik regulujący skład botaniczny runi. *Folia Universitatis Agriculture Stetinensis* 197(75), 267–270.

Rogalski M., Kryszak J., Kardyńska S., Wieczorek A. and Biniaś J. (2000) Wpływ odchodów pasących się zwierząt na zróżnicowanie składu botanicznego runi. *Zeszyty Naukowe AR w Krakowie* 73, 263–268.

Warda M. and Rogalski M. (2004) Zwierzęta na pastwisku jako element krajobrazu przyrodniczego. Annales UMCS E, 59(4), 1985–1991.

# Session 5

# Socio-economical aspects of grassland

# Competitiveness of the dairy sector at farm level in the EU

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#### Abstract

Agriculture, and the dairy sector in particular, has entered a phase of considerable change. Traditional EU policy supports are now less prevalent due to recent CAP reform and the most significant policy in the dairy sector, the milk quota, is to be removed in 2015. In light of these changes this paper examines the competitiveness of the milk sector at farm level for a selection of EU Member States. The analysis is based on the EU Farm Accountancy Data Network (FADN). Comparison is made between the main dairy producing and exporting countries in the EU15 using the grass-based production system prevalent in Ireland as a point of comparison with systems elsewhere in the EU.

Keywords: competitiveness, dairy sector, farm level, policy, economy

#### Introduction

The issue of the competitiveness of the EU dairy sector has been addressed in a number of studies conducted over the last 20 years (Boyle *et al.*, 1992; Fingleton, 1995; Vard, 2001; Boyle, 2002; Thorne, 2004.). Generally these studies took place in a period when milk quotas applied in the European Union (EU), relatively stable input and milk prices prevailed and where the EU dairy sector continued to remain largely insulated from variations in world market supply and demand conditions. Increasingly, it is the case that these factors no longer hold true. It is therefore appropriate to return to this issue.

For the purpose of this study profitability is used as a measure of competitiveness, hence both costs and returns are considered important in determining the competitive position. The focus of the paper is at the farm level. While there are also issues of competitiveness further along the production chain, these are not considered in this study. Comparison is made between the main dairy producing and exporting countries in the EU15 using the grass–based production system prevalent in Ireland as a point of reference.

Section 2 of this paper provides a context for the study by reviewing the main developments in the dairy sector in the EU in the recent past. Section 3 of the paper examines how the competitiveness of the main players in the EU dairy sector has evolved through time, with a particular focus on the period 2000 to 2010. The paper then looks beyond the EU to make comparisons among some of the main players in the dairy sector globally. The final section details some caveats that need to be considered and provides conclusions.

## Context

Since 1983 the milk quota has constrained the development of the dairy sector in the EU. The main factor that motivated the introduction of the milk quota, income support through stable milk prices, was successful in limiting the variability in dairy farm incomes for a period. However, it can be argued that milk quotas limited national milk output, hindered the expansion of individual producers, created barriers to new entrants and therefore limited competition between producers – both within and between EU Member States (MS).

After EU milk quotas were introduced world milk production and trade in dairy products continued to grow. Over the period 1980 to 2010 the volume of dairy commodities (in milk equivalent terms) traded internationally has risen from about 25 million tonnes to 45 million tonnes, an increase of 80 percent. However, as a share of world milk production the proportion of milk that is traded internationally remains low, at less that 7 percent. By contrast about 30 to 40 percent of the global production of wheat and soybeans is internationally traded (FAO, 2012).

Over the last decade, the EU dairy sector has been overtaken as the number one player in world trade by international competitors such as New Zealand (NZ), which increased its milk production and exports by about 40 percent over the period 2000 to 2010. More recently the US has also emerged as a significant player in global dairy trade, notably in the SMP (skimmed milk powder) and whey markets.

The gap between world producer milk prices and producer milk prices in the EU and US is shrinking. Using New Zealand producer milk prices as a proxy for world producer milk prices, there has been a convergence towards EU and US producer milk prices in recent years, as shown in Figure 1.



Figure 1. Producer milk prices EU15, US and NZ Source: FAPRI

The growth in farm scale is a feature of modern agriculture internationally. Over time strong production growth has been facilitated through technological development and this has led to falling prices for many agricultural commodities. Some producers have responded to these changes by exiting agriculture and others have responded by increasing farm size to exploit economies of scale.

The number of cows on the typical farm has increased over the recent decades in most countries. The rate of increase in the average herd size has been lower in the EU than in competitor countries such as the US, Australia or New Zealand. It can be argued that partially this may be due to the rigidities created by the EU milk quota system and associated milk management tools, but lying behind this is a greater political willingness in the EU to support smaller scale, family farms than is the case in competitor countries. Figure 2 shows the evolution of the average dairy herd size in the EU, New Zealand and the US from 1990 to 2010.



Figure 2. Average dairy herd size in selected EU15 Member States, NZ and US Source: LIC, FAPRI, FADN

Much of the focus on the volatility of prices associated with agriculture in the last decade has been on the price of output. This is understandable given that changes in the price of agricultural output is of concern to policy makers and consumers as such price changes impact on the prices of food at retail level. However, there has also been a pronounced volatility in agricultural input prices in recent years, which has had an adverse impact on producers and consumers. Figure 3 shows the extent of the variability of monthly feed and fertiliser prices from 1995 to 2010 using Ireland as an example. Similar patterns for feed and fertiliser prices are found elsewhere across the EU.

Animal feed and fertiliser are the main inputs which affect the cost of milk production in grassland and confinement systems. The impact which the price of these inputs will



Figure 3. Index of monthly nominal feed and fertiliser prices in Ireland 1995–2010 Source: Central Statistic Office, Ireland

have on the cost of production will depend not alone on the extent of the price change, but also on the extent to which they are required in the production system and the capacity of the production system to adjust in order to minimise the impact that a rise in the price of an input has on per unit production costs.

While there has been a general upwards trend in these input prices over the period shown, the increase in prices has accelerated since 2005 and there was also a pronounced spike in prices in 2008. This pattern in fertiliser prices has also been observed internationally (Eurostat, 2012).

It is argued that the low input extensive grass-based production system used in New Zealand and in the somewhat similar system operated in Ireland in the EU presents advantages in times of high animal feed prices. This is because the input content of feed in these production systems is relatively low compared with confinement systems and the usage of synthetic fertiliser is carefully managed. Therefore any change in the price of such inputs has a lower impact on the cost of production than would be the case if these inputs were a larger component of production costs.

The flip side is that in these low input production systems, output per cow is low and yields can be up to three times greater in more intensive production systems that favour concentrates over grass e.g. the Netherlands. Production is also more seasonal under the (grass-based) NZ or Irish system, which makes such systems more suited to regions with a large export capacity (self sufficiency ratio), which still allows domestic consumption to be met by domestic production in the trough of the production season. These low cost systems are less suitable where the domestic market for fresh products (such as drinking milk) represents a large component of milk utilisation.

The cost advantages of strongly seasonal milk production at farm level are also offset to some degree by the higher costs such systems impose at the processing level. Principally this is due to the lower average rate of capital utilisation in these processing facilities, which need to be built to cater for peak period milk production but which only operate at this level for a limited period of the year. Processing costs would be lower if the peak and the average level of production over the season were similar.

Low input systems also face difficulties as the volume of the farm's output increases. To retain the production system whilst increasing the volume of output will ultimately require that either additional land is purchased or leased, or that increasing amounts of feed and fertiliser are used. Where land prices are high or where no land is available locally, the only feasible option may be to increase input usage which in turn will reduce the cost advantage of such systems relative to more intensive feed-based systems.

As low input systems typically require more land per unit of output, this has implications for the opportunity costs associated with the calculation of the full economic costs of production, as will be observed in Section 3.

Given that low input systems produce relatively low yields, the volume of labour input per unit of output can be high. Low levels of productivity can be an issue when labour costs or land prices are rising, as labour costs and land prices can have greater implications for the total costs of production on a low input system than in more intensive production systems. Cross country comparisons are complicated by limitations in data availability and, in particular, data consistency. A common reporting structure exists to allow the comparison of countries in the EU15, the EU Commissions' Farm Accountancy Data Network (FADN) and shortly this will encompass the EU27. For comparisons globally the Dairy International farm Comparisons Network (IFCN) can be used.

## Measurement and methods

In this section indicators of cost competitiveness and partial productivity among specialist milk producers in selected EU15 MS are defined. The countries examined are those with a strong tradition in dairy production, namely: Belgium, Denmark, France, Germany, Ireland, Italy, the Netherlands and the UK. Country specific information on the extent of intra–EU trade of milk products is not available, but over 85% of the EU production of butter and cheese is accounted for by the countries specified.

Understanding the different measures of cost is vital in assessing competitiveness. While it is possible to focus on the cash costs of production alone, the wider definition of economic costs, which also includes an estimated value for own land, family labour and non–land assets, is important. To measure competitiveness, costs can be expressed relative to output value, per unit of product or per hectare.

The FADN is the main source of the data used for this analysis. Data analysis was confined to specialist dairy farms as defined by FADN (Farm Type 411), on which the standard gross margin from dairying accounts for at least two-thirds of the farm total gross margin. This allows a greater degree of accuracy in the allocation of costs (which are presented on a whole farm basis in the FADN) to the dairy enterprise than would be the case if all farms with a milk enterprise were selected for analysis (Fingleton, 1995).

FADN data can be use to calculate partial productivity indicators for the dairy sector. The measures relate to animal, land and labour productivities, namely,

- milk yield per cow (kg)
- milk solids per cow (kg)
- stocking rate (LU ha<sup>-1</sup>)
- milk production per hectare (kg)
- milk solids per hectare (kg)
- milk production per labour unit (tonne).

In turn two separate measures of cost comparisons are used for specialist dairy farms:

- total costs as a percentage of dairy output;
- total costs per unit volume of milk production.

The value of dairy output was calculated as milk receipts plus dairy calf sales. Whole farm calf sales were apportioned to the dairy enterprise based on the ratio of dairy cows to other cows. Due to data constraints it was only possible to include a value for dairy calf sales off farm. It was not possible to impute a charge for calves from the dairy enterprise transferred to the beef enterprise.

The examination of the costs of milk production on a raw milk volume basis fails to account for possible variation in milk constituents between different countries. Such approaches are biased in favour of countries where the levels of milk constituents are relatively low. To overcome this bias the unit of measure is the costs per kilogramme of milksolids i.e. butterfat plus protein (Fingleton, 1995). Average fat and protein percentages for each country were used to convert the milk volumes obtained from the FADN data into the equivalent quantities of milksolids.

In the FADN all costs are specified on a whole farm basis. However, in this study methods were devised whereby the costs were apportioned to the dairy activity. See Table A.1 of Donnellan *et. al* (2011) which outlines the allocation keys used for the purpose of defining costs associated with the dairy enterprise. This allocation method is based on that used by Fingleton (1995) and further developed in a similar study carried out by the FADN (Vard, 2001).

The IFCN data network is a world-wide partnership that links agricultural researchers, advisors and farmers to create a better understanding of milk production and the costs and returns of production world wide. The cost calculations within the IFCN network are based on individual representative farms, rather than on the results from stratified random samples of the population as is the case with FADN data. None the less IFCN data provides a source of data which can be used to examine the relative international competitiveness of 'representative' milk producers across the world. Data is assembled and analysed using a common methodological framework. Like the methods outlined previously for FADN data, IFCN data also presents costs as total 'cash' costs, which consists of expenses from the profit and loss account and total 'economic' costs with opportunity costs calculated for farm-owned factors of production (family labour, own land, own capital).

## Results

EU15 comparison: The results for the dairy enterprise are presented in two sections: (i) partial productivity indicators and (ii) comparative costs of production. In Figure 4 and Figure 5 below the partial productivity indicators identified above are outlined for the eight EU countries compared in this analysis. The results are presented for all specialist dairy farms in the sample, weighted to present population means. The results presented here for each of the countries are the average for the years 2005 to 2007. The countries under study are indexed relative to the grass-based production system used in Ireland. This means that index values greater than the value of 1 given to Ireland indicate higher levels of productivity relative to the grass-based system used in Ireland. Figure 4 shows that average milk yields per dairy cow were much lower in the grass-based system in Ireland relative to the other EU Member States included in the analysis.



Figure 4. EU partial productivity measures: milk yield, milk solids per cow and stocking rate (average 2005–07)

Average yields in the Netherlands and Denmark were substantially higher than in the other countries in the analysis. In addition, milk solids per cow were substantially lower in Ireland than in the Netherlands and Denmark, where levels were approximately 100 per cent higher than Irish dairy herds. The levels of land productivity in the Netherlands and Denmark were relatively high, with stocking rates 25 per cent and 27 per cent

higher than the grass–based system in Ireland. Only France and Germany had stocking densities lower than Ireland, with densities 32 per cent and 2 per cent, lower than in Ireland. Figure 5 shows partial productivity measures for milk production and milk solids per ha and milk production per labour unit. The combination of the relatively low stocking densities and milk yields found in Ireland are aggregated in the next two measures of productivity –milk production per hectare and milk solids per hectare. Denmark and the Netherlands showed the highest levels of milk production per hectare and milksolids per hectare, whereas among the countries under study these measures were lowest in Ireland and France.

The Netherlands and Denmark again exhibited figures well in excess of the other countries examined, with milk production per hectare 79 per cent higher in Denmark and 77 per cent higher in the Netherlands compared to Ireland. Furthermore, milksolids per hectare were quite substantially higher in other countries relative to Ireland, with levels in Denmark and the Netherlands in excess of 100 percent above Ireland.

The final partial productivity measure shown in Figure 5, milk production per labour unit, was again highest in the Netherlands and Denmark, with levels in the UK also relatively high. Italy and France were the countries that exhibited the lowest average level of labour productivity, but the average levels of labour productivity in Germany and Belgium were not much higher.



Figure 5. EU partial productivity measures: production, milk solids per ha and production/labour unit (average 2005–07)

All of the results presented in Figure 4 and Figure 5 are based on all specialist dairy farms in the sample, however these results are influenced by distribution differences in the sample farms included in the FADN survey for the different countries (Fingleton, 1995). For this reason the productivity indicators for farms with 50–99 cows were also examined in each of the EU15 countries. However, despite the variations in sampling procedures adopted in the FADN survey, there was no evidence of pronounced differences in average productivity levels between the 50–99 cows sub sample and the whole sample. In general, the productivity rankings between the countries were similar in the two samples, but the relative differences between the countries tended to be reduced in the more homogeneous sample of the 50–99 cow farms. This was particularly evident in the land and labour productivity measures, where the large disparities between the countries in the average sample of farms were reduced in the sample of 50–99 cow farms.

The first measure of comparative costs of production used in this analysis was costs as a percentage of total dairy output. This approach to measuring competitiveness seems appropriate given that volatility in input and output prices has been a significant feature of the EU dairy sector in recent years and with policy interventions likely to be less prevalent in the future this volatility is likely to remain a significant feature of the sector.

Figure 6 and Figure 7 below show the average cost relative to output measure for the threeyear period (2005–2007) using FADN data and an estimated ratio for the three-year average (2008–2010), for each of the selected countries, for all specialist dairy farms in the FADN sample.



## Cash Costs Imputed Costs

Figure 6. Economic and cash costs for specialist milk producers in EU (2005-2007)



#### □ Cash Costs ■ Imputed Costs

Figure 7. Estimates of economic and cash costs for specialist milk producers in EU (2008-2010)

Figure 6 and Figure 7 show that Belgium had the lowest cash costs as a percentage of output, with the cost structure in Italy and Ireland only slightly higher. The highest cash costs as a percentage of output were experienced in Denmark. Further analysis of the specialist dairy farms that had between 50–99 dairy cows did not show substantial deviation from these results.

When total economic costs were considered, the competitive position of the countries examined in the study changed. Notably, the competitive advantage of grass-based Irish producers deteriorates when all imputed charges for owned resources are taken into consideration.

Total economic costs as a percentage of output were highest in Denmark where costs were 111 per cent of the dairy enterprise output (2008–2010). Ireland followed with the second highest total economic costs at 103 per cent of output (2008–2010). The most significant imputed cost that contributed to the relatively high total economic costs experienced by grass-based production in Ireland over the period was the imputed charge for owned land. This was due to the relatively high rental charge used to calculate the imputed value for owned land, coupled with high levels of land ownership in Irish dairy production. Note that for FADN accounting purposes if land is rented by the operator it appears as a cash cost, whereas if land is owned by the operator is does not represent a cash cost and instead it appears as an imputed cost in the wider economic cost definition. The relatively low stocking rates and milk yields per hectare on grass-based Irish dairy farms over the period also must be considered as a contributing factor in the high economic costs of grass-based production in Ireland.

The lowest total economic costs were experienced in the Netherlands, where 20 per cent of dairy output remained as profit for dairy producers on average over the period i.e. total economic costs were just 80 per cent of total dairy output.

When total economic costs were considered as a percentage of output for specialist dairy farms with 50–99 dairy cows, the rank order also changed from the average position for the total sample shown in Figure 6 and Figure 7. Total economic costs for the 50 to 99 cow sub sample of farms were generally substantially lower than for the average farm across the full sample. For example in the case of Ireland, total economic costs as a percentage of output were reduced by just over 20 percent, when the larger size farm was compared to the average size farm. However, Denmark and Ireland still had the highest total economic cost producer for farms with 50–99 dairy cows.

The second measure of comparative costs and returns used in this analysis were costs (both cash and economic) per kg of milksolids produced. This measure takes into account the variation in the milk constituents (fat and protein) between different countries. The average cash and economic costs per kg of milksolids produced, over the period 2005 to 2007, and 2008 to 2010, for each of the countries in the analysis are presented in Figure 8 and Figure 9. Figure 8 and Figure 9 show that the consideration of the milksolids produced, has a considerable influence on the competitive position of the countries examined. The magnitude of the differences was much less between the countries when milksolids production rather than the volume of milk production was used.

Based on total cash costs per kg of milksolids produced, Italy and Denmark still exhibit very high costs. On a cash costs basis, per unit of milk solids, Ireland has costs approximately 6 per cent below the average of all countries examined (2008–2010). On a total economic cost basis, the Netherlands had the lowest costs per kg of milksolids, Ireland had the third highest costs, with only the Denmark and Italy experiencing higher units costs. When the sub sample of farms with 50–99 dairy cows were examined cash costs did not change noticeably but economic costs were reduced significantly for these farms. Furthermore, total economic



□Cash ■Imputed

Figure 8. Cash and economic costs per kg milksolids - average (2005-2007)



Cash Imputed



costs per kg of milk solids, for larger than 'average' sized grass-based systems of production, such as larger sized farms in Ireland, were below the average of all countries examined. Further analysis of the cost structures of the competing countries (Donnellan *et al.*, 2011) gives an indication of the sources of competitive advantage and disadvantage for grass-based producers in Ireland over their EU counterparts. As was discussed above, the cash cost structure for grass-based Irish milk producers over the period under examination was relatively low compared to the other countries that were examined. The cost components analysis indicates that this was associated in particular with relatively low costs for seeds and plants, crop protection, purchased feedstuffs and machinery. However, these relatively low costs were offset, in particular, by high costs for fertiliser and imputed charges for owned land. These cost components provide some indication of the sources of competitive advantage

and disadvantage associated with milk production from grass in Ireland over the period relative to competitors in the rest of the EU15.

Global comparisons: The comparisons based on the IFCN data are presented on a 'two-tiered' basis (i) cash costs and (ii) economic costs compared to milk price received. The comparisons show typical dairy farms in Ireland (as a reference point for the EU15) along with Poland, New Zealand and various states in the USA. Actual data from the IFCN were available to 2008 and was updated to 2010 to reflect input and output price movements. The most recent three year average for 2008–2010 is presented below. The US dollar was chosen as the common currency measure for all countries' results and all the remaining figures in the paper are measures expressed on US\$ per 100 kg milk (ECM).

In Figure 10 the first measure used for comparison is cash costs and milk price per 100 kg of milk (average 2008–2010). This measure indicates how well placed typical farms would be if prices or costs moved adversely relative to each other, especially in the short to medium term. This measure shows that the typical Irish dairy farm appears to have a relatively good position compared to most other dairy countries examined in the analysis with only New Zealand showing comparable profit margin levels (i.e. margin over cash costs). The typical US farms in Wisconsin, California and Texas and the larger size typical farm in Poland were in intermediate positions in terms of margin over cash costs. But the results from typical farms in Idaho and the North East of the US and the small family run farm in Poland were reported to having significantly higher cash costs per kg of milk than farms in competing countries. This meant that for the years 2008–2010 the aforementioned typical farms struggled to maintain a positive margin over cash costs. Therefore, those farms would be most vulnerable to a cost/price squeeze.



Figure 10. International comparison of cash costs of milk production and milk price: 2008–2010 Source: IFCN data (2008) and authors' own estimates (2009 & 2010) Note: IE – Ireland, NZ – New Zealand, PL– Poland, WI – Wisconsin, CA – California, ID – Idaho, TX – Texas, NY – New York

Figure 11 shows that Ireland's comparative position deteriorated very substantially when total economic costs were compared outside of the EU15. The average size Irish dairy farm (IE–48) had one of the highest total economic costs per kg of milk for the years 2008–2010, with only the average and small typical farms in Poland and the small typical farm in the North East US experiencing higher per unit total economic costs. However the larger size typical Irish dairy farm (IE–110) did exhibit somewhat lower total economic costs than the average size Irish farm, appearing about mid way in terms of total economic costs amongst



Figure 11. International comparison of economic costs of milk production and milk price: (2008–2010) Source: IFCN data (2008) and authors' own estimates (2009 & 2010) Note: IE – Ireland, NZ – New Zealand, PL– Poland, WI – Wisconsin, CA – California, ID – Idaho, TX – Texas, NY – New York

the typical farms examined. The lowest per unit total economic costs amongst the countries examined were shown to be in New Zealand for the period 2008-2010. (Further analysis in Donnellan *et al.*, (2011) also showed that total economic costs per unit of milk were relatively low in Argentina and Australia).

In terms of margin over total economic costs, the top ranking position goes to typical farms in New Zealand and the US (California and Texas).

## Caveats

In the context of assessing how competitiveness of the EU dairy sector at farm level might change in the future, there are very many issues which could each individually merit a separate study. While it has not been possible to give these issues detailed consideration in this paper, it is important that they are recalled when assessing the competitive position of the sector. In this section issues which are likely to affect the sector's future competitiveness are explored. The section ends with some conclusions.

The removal of the milk quota is expected to present new opportunities for expansion of the dairy sector in parts of the EU. However, studies such as Binfield *et al.* (2008) and Bouamra-Mechemache *et al.* (2008) have concluded that expansion in production is less likely to occur in some parts of the EU following quota elimination. It is conceivable that milk quota removal could allow scale economies to be exploited and thereby improve the competitive position of the dairy sector in some EU Member States relative to competitors elsewhere in the EU.

However, the removal of milk quotas does not mean that the dairy sector will not face other constraints, notably environmental constraints. For example, in the case of the dairy sector in Ireland, a particular concern is the high proportion of greenhouse gas (GHG) emissions that come from agriculture including those from dairy production.

While no decisions have been taken in respect of the CAP reform in 2013, the outcome could see a change in the total budget for the Single Farm Payment (SFP) and may include

a rebalancing of payments between EU Member States and for some EU Member States this would mean a move away from the historical payments system where moves to a flatter payments system have not already taken place. This would involve a redistribution of the SFP between farms. While the SFP is categorised as a decoupled payment it may affect producers' attitudes to risk, their production decisions and the volatility of their farm income. At this point it is not possible to draw any firm conclusions as to the impact potential changes to the SFP would have on the competitive position of the dairy sector across the EU. Progress in the WTO negotiations has been very slow in recent years. However, this does not mean that negotiations aimed at liberalising trade have been abandoned. Lack of progress through the WTO mechanism has seen an increase in bilateral negotiations concerning trade. In the context of the next 5 to 10 years it is not possible to rule out reductions in trade barriers between the EU and third countries which could expose the EU dairy sector to greater competition on its home markets.

In some EU Member States the dairy exports to third countries are of considerable importance. Increased milk production in some EU MS would mean that given the mature character of the EU market for dairy products, the third country export orientation of the dairy sector in parts of the EU would further increase. In such circumstances, trade agreements could have a negative impact on dairy product markets and producer milk prices. This could have greater adverse consequences for the competitive position of the dairy sector for dairy exporters in the EU relative to competitors elsewhere in the EU with only a limited dependence on markets outside the EU.

The growing requirement to use renewable energy sources in the EU will generate an increased demand for biofuels in this decade. This will increase competition for crops as an energy feedstock rather than as an animal feed. The result may be a permanent increase in feed prices and a relative increase in the prices of animal feed relative to grass. Grass–based milk producers may be better placed than those in competitor countries to deal with such an increase in feed prices.

#### Conclusions

The results of this study indicate that over the last 15 years there has been only a limited change in the competitive position of the main dairy producing and exporting nations in the EU. On a cash cost basis the grass-based dairy system (such as that in Ireland) compares favourably within in the EU. However, consideration of imputed costs allow for an evaluation of competitiveness based on total economic costs. In this context the grass-based dairy system, such as that in Ireland, would appear to be at about the average among the competitor EU15 MS dairy sectors examined.

While grass-based systems can have low cash costs of production, they may also be characterised, by relatively low productivity in terms of labour, milk yields and constituents. Hence, imputed land costs are high in the grass-based Irish system and are a key reason why the competitive position of the grass based Irish dairy sector is less favourable on a total economic cost basis when compared with key EU competitors.

Taking Eastern Europe and countries outside the EU into consideration, the position in relation to cash costs is similar to that witnessed within the EU15. The margin over economic costs also follows a similar pattern to that witnessed within the EU15, with the opportunity costs on grass based system being higher than those on farms reliant on a higher proportion of concentrates relative to grass in the diet.

#### References

Binfield J., Donnellan T., Hanrahan K. and Westhoff P. (2007) CAP *Health Check Analysis: Impact on EU Milk Quota Expansion*, Teagase, Carlow, Ireland.

Bouamra-Mechemache Z., Jongeneel R. and Réquillart V. (2008) Impact of a gradual increase in milk quotas on the EU dairy sector. *European Review of Agricultural Economics* 35, 4, 461–491.

Boyle G.E, Kearney B., McCarthy T. and Keane M. (1992) *The Competitiveness of Irish Agriculture*, Allied Irish Banks and the Irish Farmers Journal, Dublin.

Boyle G.E. (2002) *The Competitiveness of Irish Agriculture, Report for the Department of Agriculture and Food*, The Irish Farmers Journal, Dublin.

Conner K.R. (1991) A Historical Comparison of Resource-based Theory of Five Schools of Thought Within Industrial Organisation Economics: Do We Have a New Theory of the Firm?. *Journal of Management* 17 (1), 121–154.

CSO, (various years) http://www.cso.ie/.

Donnellan T., Hennessy T., Keane M. and Thorne F. (2011) *Study of the International Competitiveness of the Irish Dairy Sector at Farm Level*, Rural Economy Research Centre, Teagasc.

Eurostat (2012) Eurostat Statistical Database, http://epp.eurostat.ec.europa.eu/portal/page/portal/statistics/ search\_database

FADN (2003) Farm Accountancy Data Network, http://europa.eu.int/comm/agriculture/rica/index\_en.cfm [accessed 20th June 2011].

FAO (2012) FAOstat, www.faostat.org [accessed 12 April 2012].

Fingleton W.A. (1995) 'Comparative Costs and Returns for milk production in EU countries', paper presented at the Annual Conference of the Agricultural Economics Society of Ireland, Dublin, October 1995. Thorne F. (2004) Measuring the Competitiveness of Irish Agriculture (1996–2000). Rural Economy Research Series No. 9, Teagasc, Dublin.

Thorne F. and Fingleton W. (2006) Examining the Relative Competitiveness of Milk Production: An Irish Case Study (1996–2004). *Journal of International Farm Management* 3, 4 December 2006.

Vard T. (2001) *Costs of Production for Milk in the European Union Period 1989/90–1998/99 (revised methodology)*, RICC1331 En, Community Committee for the Farm Accountancy Data Network.

# The role of grassland in rural tourism and recreation in Europe

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#### Abstract

Grasslands are an essential element of sustainable farming systems and are acknowledged as having economic, ecological, social and cultural roles. In many areas raising animals in a grassland-based system is providing farmers with a decent income while in others the only way for farmers to stay in agriculture is to 'cultivate' tourists through agritourism and ecotourism. Agritourism is a particular form of rural tourism, i.e. farmers provide on-farm activities connected to farming. Grassland in a diversified landscape provides an added value for tourists and is the most important resource for tourism development having a greater aesthetic and recreational potential than uniform agricultural areas both in lowlands and mountains. Consumers often perceive food products from extensive and organic grassland-based agriculture as of higher quality. Nature-based tourism (ecotourism) where people visit rural areas characterized by high species and habitat diversity is also an important source of additional income to farmers. Ecotourism generates many economic benefits for local communities and activities related to farming are more attractive, such as the direct sale of products labelled as coming from Natura 2000 sites. Tourism may also have negative impacts when the number of tourists is large or the resources are overused.

Keywords: grassland, multifunctionality, rural tourism, agritourism, ecotourism

#### Introduction

Grasslands sensu lato are among the largest and most important habitat types in the world covering 40.5% of the Earth's mass (Suttie et al., 2005; Gibson, 2009). In Europe there are various types of grasslands, ranging from almost desertic types in south-east of Spain through steppic and mesic types to humid grasslands/meadows in the north and north-west. Almost all European permanent grasslands could be defined 'semi-natural grassland' because they are more or less modified by human activities. Some more natural 'permanent grasslands' are present over the 'treeline' of mountains and in areas with extreme natural conditions (climate, topography, soil) (EC, 2008a). In the EU-27 grasslands and rangelands represent 33% of the Utilized Agricultural Area (UAA) (Peeters, 2009; Osterburg et al., 2010) and, according to official Agricultural Statistics (Eurostat, 2010), 13% of the EU-27 territory. While more than 45% of the land in Ireland and the United Kingdom is used for seminatural and permanent grassland, extreme northern and southern countries (e.g. Finland and Cyprus) have less than 1% of their land under permanent grassland. This is determined by the high variability of ecological and cultural conditions, and diverse production systems. Grasslands may be semi-natural, permanent or temporary and can be grazed, cut or used both for grazing and cutting. Pflimlin et al. (2005) identified seven forage and livestock regions in the EU-15 i.e. (i) northern regions (Scandinavia), (ii) wet mountain regions (e.g. Alps, Pyrennes), (iii) mediterranean regions in fertile plains and valley as well as in dry mountain rangelands, (iv) grassland regions with permanent grassland dominant in the UAA (e.g. Ireland, north-east of France), (v) grassland and maize regions (e.g. north-west
of Germany), (vi) forage crop regions with temporary grassland plus maize (e.g. Brittany, Flanders), (vii) arable land and livestock regions (e.g. Po Valley).

In the EU, grasslands strongly declined in recent decades and only few Member States (i.e. Denmark and Portugal) managed to stop this negative trend (FAO, 2006). This reduction of grassland area was mostly in favour of the production of annual crops and fodder maize. In mountainous and mediterranean areas marginal grassland has tended to be abandoned or intensively used, causing various environmental problems.

In Europe semi-natural grasslands are an essential element of sustainable farming systems and are acknowledged as having a multifunctional role. Mountain grasslands are highly multifunctional, because the benefits of ecosystem services accrue to both mountain and lowland populations, maximizing highland-lowland complementarities (EEA, 2010a). From the production point of view grasslands offer forage for domestic animals and promote the value of related products. From the ecological point of view, grassland management has an impact on ecosystem processes modifying biodiversity, air, soil and water. From the social and cultural point of view, grasslands help to maintain the viability of rural communities as an important source of employment, improving rural tourism and recreation (Kemp and Michalk, 2007; Gibon, 2005).

Grasslands are the source of feed for animals and the importance of forage quality for animal origin products is well known (Piasentier and Martin, 2006; Bovolenta *et al.*, 2011). Grassland based agriculture is recognized to provide not only food and fibre but also benefits with regard to sustainable agriculture (UNCED, 1992). Important ecosystem services provided by grassland have been identified by the Millenium Ecosystem Assessment (MEA, 2005). Grassland ecosystems represent the most important part of biodiversity, i.e. they offer ideal conditions for diversity of habitats and species, and are very important for birds and invertebrates. The Habitats and Birds directives are the main legislation acts ensuring the protection of Europe's grasslands. In 2006, the EC adopted an Action Plan that defines priority actions to halt biodiversity loss, i.e. to the conservation and wise use of grasslands, particularly through the maintenance of extensive systems. In addition to species conservation and habitat protection, grassland biodiversity can contribute to enhanced value of traditional agricultural products and to non-commodity outputs, ecosystem functions and resilience to environmental perturbation (Hopkins and Holz, 2006).

The Common Agricultural Policy (CAP) of the EU and the increasing emphasis on payments for public goods (positive externalities and ecosystem services) is expected to become further strengthened with the next 2014–2020 CAP reform. Payments for Ecosystem Services (PES) should also be a tool to provide additional income for alternative land uses or particular practices at farm level. PES projects are usually classified not specifically for agriculture but rather as schemes to protect water, carbon sequestration, biodiversity and landscape beauty or as PES for connected services. These initiatives are sometimes found in agricultural policies or agri-environmental schemes (Ottaviani, 2011; Lampkin, 2011). Some PES schemes are prepared to protect landscape beauty related to agriculture and grassland landscape aesthetics when they involve rural amenities (FAO, 2007).

Although agricultural policies and agri-environmental schemes give the possibility for European farmers to stay in agriculture, for many of them, the only possibility is to diversify and improve their incomes, either through new enterprises on-farm or through off-farm integrative employment. Many farmers are exploring the possibility of switching to rural tourism and 'cultivating' tourists rather than only crops or pastures. Rural areas are in a process of change due to the impact of globalisation that has changed market conditions and

orientations for traditional products. Rural areas are difficult to define since criteria used by European Countries are different.

In this complex situation rural tourism has been the subject of many debates in the literature without arriving at any firm consensus (Pearce, 1989; Bramwell, 1994). Rural tourism involves activities which have their own environment and culture, and encompasses all the activities which may be carried out in a rural environment. Tourism is called 'rural' when the rural culture is a component of the offered product. The expressions 'rural tourism', 'agritourism' and 'ecotourism' are often improperly used as synonyms. 'Grassland tourism' is favoured in the 'forage and livestock regions' where more semi-natural and/ or permanent grasslands are present.

#### Agritourism and grassland

Agritourism is a particular form of rural tourism i.e. farmers provide on-farm activities connected to farming (food production, milking, harvesting, etc.). Tourists may buy products and spend from just a few hours to their whole holidays on the farm, in typical rural buildings. Different forms of agritourism have developed in many regions and therefore it is hard to find common characteristics for all countries. Agritourism in many countries consists in turning farm buildings into rustic lodging facilities. Sometimes, 'cultivating tourists' on the farm means a simple style of life like roll out sleeping bags and sleeping on the floor. Agritourism refers in general to farm stays but often tourists may stay in very comfortable rooms or apartments at the level of the best city hotels. Very often, agritourism in grassland and picking mushrooms, feeding animals, riding horses, having a hay bath etc. Agritourism can be in general defined as 'a range of activities, services and amenities provided by farmers' (Beus, 2008). It may be concluded that in all these situations grasslands are the core element for agritourism activities.

The importance of agritourism has been steadily increasing in many European countries. The growth of agritourism is difficult to quantify because relatively few countries collect precise statistics. Already in the mid-1990s, 12 European countries (Belgium, Denmark, Greece, Netherlands, Portugal, Spain, Ireland, Great Britain, Italy, Austria, France and Germany) had more than 100 000 farm enterprises involved with different tourism activities (Krizman-Pavlovic, 2001). According to Roberts and Hall (2001) tourism in rural areas makes up 10 to 20% of all tourism activities and every year 23% of European tourists choose the countryside as a holiday destination. Agritourism development is also related to the presence of infrastructures and presence of local products but the attractiveness of rural areas for tourism and recreation can be also related to the scenic beauty of rural landscape, e.g. flowering meadows or animals grazing on pastures. Through agritourism many farmers have the opportunity to diversify activities, to give added value to products and protect the environment. In many situations tourists have the opportunity to learn crafts and skills, to enjoy traditional home-made food and drinks. Tourism is often a strategy for farms to employ female labour and to increase income. This is often frequent and desirable, especially in less favoured areas (LFA) of Europe (Ibery et al., 1997).

Traditional and local products can be enhanced by farm labels or by territorial labels like Protected Denomination of Origin (PDO), Protected Geographical Indication (PGI) and traditional local products or territorial brands. Also organic products are very attractive for tourists. The EU introduced in 1991 legally binding requirements for organic farming and food production, which helped to develop a fast growing market for organic products and recommended the use of forage coming from local grasslands. The organic area of the total UAA is around 4% in the EU-27, with a slight upward trend and a wide variation between the countries is present i.e. Austria, with 16% of the UAA has the highest share. Italy accounts for nearly 9% and the Czech Republic has the highest percentage (over 8%) among the countries which joined EU in 2004. Pastures and meadows occupy a high proportion of the land e.g. in Ireland 96% of the fully converted organic area, in Czech Republic 92%, in the United Kingdom more than 70%, in Poland 52% and in Italy 23% (http://www. organic-world.net/statistics-eurostat.html). Many varieties of plant and livestock products are produced and processed organically and find their way to consumers via different market channels, but for tourists especially via direct market on farm or farmers' markets. In 2004, the EU set out its overall organic policy in the European Action Plan for Organic Food and Farming (http://www.orgap.org) and the dual role of organic production was recognised: i) it delivers public goods, environmental benefits but also rural development and animal welfare; ii) it creates a specific market responding to consumers demand for products produced by using natural substances and processes. Organic production is an overall system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources (i.e. grasslands), low input and high animal welfare standards.

Grasslands have a positive impact on tourist demand and many studies have examined the visual preferences of tourists for different types of landscape. The rural diversified landscape, with semi-natural and natural grasslands, is a most important resource for tourism development having a greater aesthetic and recreational potential than uniform agricultural areas both in lowlands (Junge *et al.*, 2011) and mountains (Hunziker *et al.*, 2008).

In research by Howley et al. (2012), a survey of 430 individuals living in Ireland demonstrated that people believe the production of high quality food is the most important function of agriculture but at the same time the highest value for multifunctional aspects of agriculture is to relax and enjoy the scenery in the countryside, kept in a good environmental state. The use of countryside for recreational activities (e.g. walking, hiking, fishing) was also very important for tourists. The perception of landscape attractiveness showed that the landscape with the highest preference was horses in an open grass-covered field, followed by a run down farm building surrounded by trees and a grass covered field with hills in the background. Sheep and cattle grazing on open grassland in a traditional landscape type were very highly preferred to a landscape type with intensive farming. These results were confirmed in other situations where grassland is the dominating landscape type (Bishop, 2005). In the lowland, citizens appreciate grazing animals in the landscape, for emotional reasons and because they associate grazing with high animal welfare. In a paper by Boller et al. (2009), grassland associated with grazing animals in the highland areas and in some semi-arid areas (e.g. the Iberian Dehesa ecosystems) were shown to promote agritourism, offering an extra income for the inhabitants.

Grassland is the most important aesthetic element of the landscape also for people living in areas where it is not dominant. In an Interreg programme cooperation project between Italy and Slovenia (Parente and Simonetti, 2008), the relationships between landscape, cultural heritage, and economic value of rural landscape were analyzed. The results of 400 interviews showed that between the elements which characterize the rural landscape, the most important were grassland, followed by streams, woodland, orchards and historical buildings, etc. The same research showed also that people recognize the environmental and social benefits provided by this landscape and express a willingness to pay (WTP) of 25.59 Euros ha<sup>-1</sup> year<sup>-1</sup>. Also the results from a research on hedonic pricing analysis (Van Huylenbroeck *et al.*, 2006) indicate that landscape features associated with agriculture such as meadows and grazing cattle, positively influence the demand for rural tourism and have a positive impact on the price the tourists are WTP for rural accommodation. This is also illustrated by the adverse impact of perceived negative externalities from agricultural production in Flanders (Vanslembrouck *et al.*, 2005), such as intensive maize cultivation, and a positive impact of meadows and pastures.

In Switzerland the Swiss Confederation defines that part of the role of agriculture is the maintenance of rural employment and cultural heritage. An area eligible for ecological compensation include semi-natural habitats, such as extensively cultivated meadows and pastures, hedges and woods, traditional orchards, ponds and stonewalls. Farmers receive an ecological compensation for extensive meadow-land, natural field margins and permanent flowery meadows. Farmers receive annual payments from the canton authorities for the adoption of these specific agronomic practices (Vermont, 2005).

In England, 23% of farms provide some type of commercial leisure service enterprise, such as fishing, nature trails, picnic sites, etc., while 24% of English farms provide overnight accommodation and/or catering (Turner and Winter, 2003).

In France, hikers and cyclists can follow a network of trails around the country that leads from farm to farm, e.g. the Limousin region in central France, a land of rolling hills covered with lush meadows with cattle, sheep, and goats, where tourism still plays only a small role in the local economy, and where visitors can enjoy a peaceful and quiet vacation away from crowded tourist destinations (Poelz, 2007).

In the Netherlands, where agriculture is managed intensively, there are also examples of protection of the agricultural scenic beauty, e.g. in the eastern part of the country where the landscape is characterized by a mosaic of small-size field plots and is appreciated by tourists. The Dutch Government established a 'landscape fund' to reward farmers for the preservation of this landscape (Almasi, 2005). In Greece, PES schemes supported by the CAP rewards farmers for conserving a region (Anfissa) of 6 000 hectares where old olive trees are grown (FAO, 2007).

#### Ecotourism and grassland

Ecotourism, is a nature-based form of tourism where people visit rural areas managed through sustainable practices and characterized by high species and habitat diversity.

The most widely used and recognized definition of ecotourism is given by the International Ecotourism Society, i.e. 'responsible travel to natural areas that conserves the environment and improves the well-being of local people' (TIES, 1990). Consequently, the main purpose is not rural activity but rather bird and animal watching, learning about local flora and fauna, and enjoying open-air landscapes of grassland (EC, 2008a).

Ecotourism is practiced in areas that are very fragile areas in ecological, social and cultural senses. It is an important tool for the creation of additional income for farmers, i.a. for the management of grasslands in Protected Areas (PA) and in mountainous areas (EPC, 2002). In addition to the role in biodiversity conservation, PA are covering a wide range of ecosystems i.a. grasslands. PA are recognized to have ecological, cultural and socio-economic values. The Convention on Biological Diversity recognises that PA also 'provide opportunities for rural development and rational use for marginal lands, generating income and creating jobs, for research and monitoring, for conservation education, and for recreation and tourism' (CBD, 1992).

Extensive grassland utilisation is compulsory to avoid negative environmental impacts and in mountainous areas to avoid the encroachment with shrubs and trees, fire risks, and losses of biodiversity (MacDonald *et al.*, 2000; Gibon, 2005).

PA, particularly those listed in IUCN (International Union of Conservation of Nature) categories IV, V and VI (Dudley, 2008), are areas where sustainable resource use and rural development practices can be tested in partnership with a wide range of stakeholders (EEA, 2010b).

Low input farming systems (LIFS) associated with high biodiversity were defined by Baldock *et al.* (1993) as High Nature Value (HNV) farmland. Links between HNV farmland and traditional agricultural landscapes are well described in the EEA report (2004).

One of the criteria for HNV selection is Natura 2000 which is an EU wide network of nature protection areas established under the Council Directive 92/43/EEC on the conservation of natural habitats and wild fauna and flora (http://ec.europa.eu/environment).

At policy level, HNV farmland, originally developed in relation to the importance of farming practices for biodiversity, has gained importance with its selection as an indicator for the evaluation of Rural Development Plans (Krautzer *et al.*, 2011).

Of the total area designed as Natura 2000 sites, 43% is in the mountain areas, compared to 29% for the EU as a whole. Slovenia has the greatest proportion (29%) of its mountain area in these sites, followed by Slovakia (23%) and Bulgaria (19%) (EEA, 2010a). It may be argued that these countries have a high potential for the future development of ecotourism. The majority of semi-natural grasslands also in Natura 2000 sites depend on regular farming activities for their existence as open habitats. Farming and the protection of Natura 2000 sites must coexist on the same land. Activities related to farming are more attractive for tourists, e.g. the direct sale of meat, milk, cheeses, labelled as coming from Natura 2000 sites, the promotion of ecotourism linked to the discovery of nature, etc. (EC, 2008a).

A significant example of a combination of agritourism and ecotourism in a grassland area is the 'Alture di Polazzo' farm (www.parcorurale.it) in the karst plateau in Italy close to the Slovenian border (Parente et al., 2011). The farm is managed according to the general principles of LIFS (Biala et al., 2008) and is located in a Natura 2000 area and profits from EU subsidies. Farming practices are associated with high biodiversity values and the area may be defined as HNV farmland (EEA, 2010b). The grassland is grazed by domestic autochthonous breeds both for productive and recreational use. Besides lamb and beef meat production, the farm offers lodging in rooms and in a camping area. All farm products are sold to visitors or served to people at the farm tavern. Didactic aspects include farming activities (i.a. honey production, animal keeping, hay making), nature observation (i.a. bird watching, vegetation analyses) and historical education (e.g. memories and remnants of the First World War). The farm activities give many benefits, i.a. maintaining pasture ecosystem, preserving the typical karst pods for animal watering, creating wetlands to aid the recovery of local grass species and birds, improving soil and grassland biodiversity through grazing, increasing of the number of invertebrates (e.g. grassland butterflies), increasing people awareness of biodiversity through education. Direct sale and in farm use of served products (e.g. meat, vegetables, fruits, cheese, honey) is the main sources of farm income. The owner declares that his gross income is three times higher than the income possible with traditional farming.

Trakolis *et al.* (2000) proposed conservation actions in Natura 2000 sites and ecotourism initiatives in addition to existing winter ski tourism in a large alpine area of Greece (Mont Voras) arguing that reasonable presence of tourists is compatible with the management of these areas when appropriate initiatives are taken.

Plant diversity in itself is attractive to humans and is an economic argument for the conservation of biodiversity in grassland. Intensification has to be avoided in mountain areas because it may have negative consequences for tourism in these regions where grasslands are the main element of the landscape (Lindemann-Matthies *et al.*, 2010). Excessive number of tourists hiking mountain grasslands causes devastating of their natural values, destroying vegetation not only on path surroundings but also on the remote sites (Barancok and Barancokova, 2008). According to Dover *et al.* (2011), the presence of tourists may have a negative effect on biodiversity measured by butterfly population when extensive managed grasslands are progressively abandoned to concentrate livestock in the bottom of valleys. Finally, in these areas agricultural products on offer to tourists are certainly less important than ecotourists' expectations for nature-based elements. Nevertheless, the possibility to enhance production systems through specific products labelling (eco-label) in order to show their contribution to reach conservation objectives must not be neglected (Biala, 2009).

#### CAP facilities for rural tourism

Sixty percent of the population in the 27 Member States of the European Union (EU) live in rural areas, which cover 90% of the territory (http://ec.europa.eu/agriculture). The European model of agriculture and rural development consists of two pillars of the Common Agricultural Policy (CAP), i.e. agricultural market support (pillar 1) and rural development policy (pillar 2). The CAP contributes to the multifunctional dimension of the European agricultural sector aiming at the sustainable production of food and non-food products, safeguarding the countryside and providing environmental services, employment in rural areas and helping to reinforce the economic and social cohesion between groups and regions. Pillar 1 was redesigned to provide basic income support to farmers engaged in food production in response to market demands. Pillar 2 was subjected to reform in 2005, resulting in an increasingly strategic and administratively simplified approach to rural development, which focuses on three core objectives (EC, 2008b), and support for rural development in 2007–2013 is provided through the European Agricultural Fund for Rural Development (EAFRD), which allocates funding to Member States through a variety of measures organized as follows: a) Axis 1 - Improving the competitiveness of the agriculture and forestry sector; b) Axis 2 - Improving the environment and the countryside through land management; c) Axis 3 – Improving the quality of life in rural areas and encouraging diversification of economic activity. A 4th axis, called 'Leader', is based on experience with the Leader Community Initiatives and introduces opportunities for locally based bottom-up approaches to rural development.

Although there is no specific support for rural tourism and multifunctionality in the EU policy, farmers may benefit indirectly i.e. partly from pillar 1 and more from pillar 2 (i.a. axis 3) and from the 'Leader axis'. The budget allocated in pillar 2 is at the moment on average only 20% of the total CAP budget and the possible allocation of more money to pillar 2 could be a positive solution for improving the environment and the countryside through land management. Thus, it would also enhance the quality of life in rural areas and encourage the diversification of economic activities like rural tourism. To support this thesis an article by Bayfield *et al.* (2008) describes eight sites of mountain areas across countries in Europe (Austria, Czech Republic, Germany, Italy, Slovakia, Spain, Switzerland, UK) where local stakeholders examined how alternative rural funding scenarios might influence the pattern of functional land types. The current rural funding was in general unsatisfactory in various respects and reduction of funding would offer opportunities for conservation and development of mountain communities and land use. Garcia-Martinez *et al.* (2011) made a simulation to analyse possible scenarios of mountain cattle farming

systems depending on changes induced by CAP subsidies. Total subsidy decoupling caused an increase in off-farm activities and a decrease in grasslands use.

For the coming CAP 2014–2020 strong lobbies and stakeholders are pressing the EC to allocate more money on pillar 2 and 'greening' pillar 1.

Finally it has to be mentioned an important EU financial instrument (LIFE) supporting environmental and nature conservation projects (ec.europa.eu/environment/life). Between 1999 and 2006, LIFE co-funded 45 projects directly targeting grassland habitats around Europe. It has been a cornerstone of grassland conservation especially within the Natura 2000 network (EC, 2008b).

#### **Discussion and conclusions**

Grassland is an essential element of sustainable farming systems and is acknowledged as having a multifunctional (economic, ecological, social and cultural) role. European grasslands feed millions of domestic and wild animals and contribute significantly to the agricultural economy of many EU regions. Grasslands must be properly managed to sustain and maintain grass-based ecosystems and communities that depend on them. In many EU areas raising of animals on extensive, artificial and permanent grasslands is still providing farmers with a decent income with relatively little capital investment. In other areas, where grass-based agriculture is not competitive with intensive agriculture or under adverse environmental conditions, the only way for farmers to stay in agriculture is to 'cultivate' tourists and to explore other positive properties of grasslands to create a 'grassland-tourism' activity because they are valuable recreation and tourism destinations.

Tourists have different targets. For some of them the main purpose is to spend a holiday far from the stressing life of the city and enjoy from 'a range of activities, services and amenities provided by farmers' (agritourists). Others are looking for buying typical agricultural products (e.g. wine, fruits, olive oil) or products from a landscape dominated by grasslands (e.g. cheese, meat), better if extensively managed (grassland agritourists). Many people visit grassland areas mostly because they are managed through sustainable practices and characterized by high species and habitat diversity and are attracted by the birds, diverse plant life and open-air landscapes of grassland (ecotourists).

Extensive grasslands and conservation actions in Natura 2000 sites must coexist on the same land. In many cases the natural characteristics of the landscape would disappear if agricultural work or animal rearing were to cease. Activities related to farming are more attractive for tourists, e.g. the direct sale of labelled as Natura 2000 products.

In many parts of Europe grasslands define the character of the landscape, e.g. it is impossible to imagine alpine upland grasslands without the presence of grazing animals. This landscape is an attractive destination for tourists and a location for recreational activities including cultural and spiritual needs and aesthetic enjoyment.

Consumers often perceive food products coming from extensive, mountain and organic grassland-based agriculture as of higher quality. Also the conservation of the mountain grasslands has allowed the local people to live in these environments and to increase the production and sale of local products such as beef, milk and cheese.

Grassland in a diversified landscape provides an added value for tourists and many studies have showed that it is the most important resource for tourism development having a greater aesthetic and recreational potential than uniform agricultural areas both in lowlands and mountains. A landscape with animals grazing on grassland with the presence of trees and hills or mountains in the background has the highest value for people. Activities such as on-farm lodging, selling food products, bird watching, hunting and other activities generate many economic benefits for local communities. Farm-based tourism gives also the possibility, especially in many LFA of Europe, to employ female family labour and to contribute in reducing the gap between male and female employment.

Beside the positive effects of tourism, also negative impacts must be considered when the number of tourists is large or the resources are overused. The negative impacts of tourism can only be managed effectively if they have been evaluated and appropriate measures have to be created. Finally, the concerns that the countryside with the presence of tourists is losing its qualities or even more that the change of farmer's activities for agritourism may threaten farming cultures are in our opinion groundless. Rather, the farmer dealing with tourists has the opportunity to be sensitive to the expectations of other people, the possibility of creating positive experiences and increasing the respect of tourists for the environment.

#### References

Almasi A. (2005) Some issues and examples of environmental services and financing for the protection and sustainable use of water-related ecosystems in The Netherlands. In: *Seminar on Environmental Services and Financing for the protection and Sustainable Use of Ecosystems*, Geneva, Switzerland, pp. 1394–1404. Baldock D., Beaufoy G., Bennett G. and Clark J. (1993) *Nature conservation and new directions in the EC common agricultural policy*, IEEP, London, UK.

Barancok P. and Barankova M. (2008) Evaluation of the tourist path carrying capacity in the Belianske Tatry MTS. *Ekologia* 27(4), 401–420.

Beus C.E. (2008) Agritourism: Cultivating tourists on the Farm. Washington State University Extension, Washington, USA, 31 pp.

Bayfield N., Barancok P., Funger M., Sebastia T.M., Dominguez G., Lapka M., Cudlinova E., Vescovo L., Ganielle D., Cernusca A., Tappainer U. and Droesler M. (2008) Stakeholder perceptions of the impacts of rural funding scenarios on mountain lanscapes across Europe. *Ecosystems* 11, 1368–1382.

Biala K., Terres J.M., Pointereau P. and Paracchini M.L. (eds) (2008) *Low input farming systems: an opportunity to develop sustainable agriculture*. Proceedings of the JRC Summer University, European Communities, Luxembourg, 115 pp.

Biala K. (ed.) (2009) *Environmental labelling and certification initiatives in the agri-food sector – a way of marketing agricultural sustainability*. Proceedings of the JRC Summer Expert Meeting, European Communities, Luxembourg, 104 pp.

Boller B., Posselt U.K. and Veronesi F. (2009) Handbook of plant breeding. Fodder crops and Amenity Grasses, Springer Science, New York, USA, 523 pp.

Bovolenta S., Dovier S. and Parente G. (2011) Dairy production systems in the Italian alpine area. In: *Contribution of mountain pastures to agriculture and environment*. Proceedings of the 16<sup>th</sup> meeting of the FAO CIHEAM Mountain pasture Network, ACW, Swiss and ITEP, Poland, pp. 143–146.

Bramwell B. (1994) Rural Tourism and Sustainable Rural Tourism. *Journal of Sustainable Tourism* 2, 1–6. CBD (1992) *Convention on Biological Diversity*. Available at: http://www.cbd.int/doc/legal/cbd-un-en.pdf Dover J.W., Rescia A., Fungarino S., Fairburn J., Carey P., Lunt P., Arnot C., Dennis R.L.H. and Dover C.J. (2011) Land-use, environment, and their impact on butterfly populations in a mountainous pastoral landscape: species richness and family-level abundance, *Journal of Insect Conservation* 15, 523–538.

Dudley N. (2008) *Guidelines for Applying Protected Areas Management Categories*. IUCN, Gland, Switzerland, 87 pp.

EC (2008a) *LIFE and Europe's grasslands. Restoring a forgotten habitat.* Office for Official Publications of the European Community. Luxembourg, 56 pp.

EC (2008b) *EU Rural Development Policy 2007–2013: fact sheet.* Directorate General for Agriculture and Rural Development, European Commission, Brussels. Belgium.

EEA (2004) *High nature value farmland. Characteristics, trends and policy challenges.* European Environment Agency reports, Copenhagen, Denmark, 32 pp.

EEA (2010a) Europe's ecological backbone: recognizing the true value of our mountains. European Environment Agency reports, Copenhagen, Denmark, 248 pp.

EEA (2010b) 10 messages for 2010. Protected areas. European Environment Agency, Copenhagen, Denmark, 9 pp.

EPC (2002) Ecotourism in Mountain Areas – a challenge to Sustainable Development. Conclusions of the European Preparatory Conference of the International Year of Ecotourism and the International Year of Mountains. Salzburg, Austria, 4 pp.

EUROSTAT (2010) *Agricultural Statistics. Main results 2008–2009*, Eurostat pocketbooks collection. 186 pp. Available at: http://ec.europa.eu/Eurostat.

FAO (2006) FAO statistical yearbook. Faostat., Rome, Italy.

FAO (2007) *The state of food and agriculture 2007*. FAO Agriculture Series n.38, FAO, Rome, Italy, 222 pp. Garcia-Martinez A., Bernués A. and Olaizola A.M. (2011) Simulation of mountain cattle farming systems changes under diverse agricultural policies and off-farm labour scenarios. *Livestock Science* 137, 73–86.

Gibon A. (2005) Managing grassland for production, the environment and the landscape. Challeges at the farm and the landscape level. *Livestock Production Science* 96, 11–31.

Gibson D.J. (2009) Grasses and Grassland Ecology. Oxford University Press, UK, 305 pp.

Hopkins A. and Holz B. (2006) Grassland for agriculture and nature conservation: production, quality and multifunctionality. *Agronomy Research* 4(1), 3–20.

Howley P., Donoghue C.O and Hynes S. (2012) Exploring public preferences for traditional farming landscapes. *Landscape and Urban Planning* 104, 66–74.

Hunziker M., Felber P., Gehring K., Buchecker M., Bauer N. and Kienast F. (2008) Evaluation of Landscape change by Different Social Groups. *Mountain research and development* 28, 140–147.

Junge X., Lindemann-Matthies P., Hunziker M. and Schuepbach B. (2011) Aesthetic preferences of nonfarmers and farmers for different land-use types and proportions of ecological compensation areas in Swiss lowlands. *Biological Conservation* 144, 1430–1440.

Kemp D.R. and Michalk D.L. (2007) Towards sustainable grassland and livestock management. *Journal of Agricultural Science* 145, 543–564.

Krautzer B., Bartel A., Kirmer A., Tischew S., Feucht B., Wieden M., Haslgrueber P., Rieger E. and Poetsch E.M. (2011). Establishment and use of High Nature Value Farmland. *Grassland Science in Europe* 16, 457–469. Krizman-Pavlovic D. (2001) Turizam na sealskin gospodarstvima. *Marketing* 3, 18–25.

Ilbery B., Bowler I., Clark G., Crockett A. and Shaw A. (1997) Farm-based tourism as an alternative farm enterprise: a case study from the Northen Pennines, England. *Regional Studies* 32(4), 355–364.

Lampkin N. (2011) Relevance of OECD Agri-Environmental measures for PES. In: Ottaviani D. and Scialabba N. (eds) *Payments for Ecosystem Services and Food Security*. FAO, Rome, Italy, pp. 45–67.

Lindemann-Matthies P., Junge X. and Matthies D. (2010) The influence of plant diversity on people's perception and aesthetic appreciation of grassland vegetation. *Biological Conservation* 143, 195–202.

MacDonald D., Crabtree J.R., Wiesinger G., Dax T., Stamou N., Fleury P., Gutiérrez-Lazpita J.A. and Gibon A. (2000) Agricultural abondonment in mountain areas of Europe: Environmental consequences and policy respone. *Journal of Environmental Management* 59, 47–69.

MEA (2005) *Ecosystem and Human well-being: Current State and Trends*. Millenium Ecosystem Assessment, Island Press, Chicago, USA, 901 pp.

Osterburg B., Isermeyer F., Lassen B. and Roder N. (2010) Impact of economic and political drivers on grassland use in the EU. *Grassland Science in Europe* 15, 14–28.

Ottaviani D. (2011) The role of PES in Agriculture. In: Ottaviani D and Scialabba N. (eds) *Payments for Ecosystem Services and Food Security*. FAO, Rome, Italy, pp. 9–29.

Parente G. and Simonetti E. (2009) Uelije Olive Oil: a transbourdary case study to support environmental labelling systems. In: Biala K. (ed.) *Environmental labelling and certification initiatives in the agri-food sector – a way of marketing agricultural sustainability*. Proceedings of the JRC Summer Expert Meeting, European Communities, Luxembourg, pp. 39–45.

Parente G., Dovier S. and Bovolenta S. (2011) Multifunctionality of karst grassland to ensure an optimal provision of public goods. *Grassland Science in Europe* 16, 556–558.

Pearce D. (1889) Tourist development. Harlow, Longman and New York. Wiley, 2<sup>nd</sup> edition. 341 pp.

Peeters A. (2009) Importance, evolution, environmental impact and future challenges of grasslands and grassland-based systems in Europe. *Grassland Science* 55, 113–125.

Pflimlin A., Buczinski B. and Perrot C. (2005) Une proposition de zonage pan-européenne pour mieux valoriser la diversité des sistèmes d'élevage d'herbivores. *Fourrages* 182, 449–475.

Piasentier E. and Martin B. (2006) From grass to fork. In: Biala K., Noesberger J., Parente G., and Peeters A. (eds) *Quality production and quality of the environment in the mountain pastures of an enlarged Europe*. Proceedings of the 13<sup>th</sup> Meeting of the FAO-CHIEAM, Udine, Italy, pp. 109–125.

Poelz V. (2007) Living in the Limousin. Transitions Abroad Magazine XXX, 5.

Roberts L. and Hall D. (2001) Rural Tourism and Recreation: principles to practice. CABI Publishing, Wallingford, UK, 237 pp.

Suttie J.D., Reynolds S.G. and Batello C. (eds) (2005) *Grasslands in the world*. FAO, Rome, Italy, 514 pp. TIES (1990) *TIES Global Ecotourism Fact Sheet*. The International Ecotourism Society. Available at: http://www.ecotourism.org.

Trakolis D., Platis P. and Meliadis I. (2000) Biodiversity and Conservation Actions on Mount Voras, Greece. *Environmental Management* 26, 145–151.

UNCED (1992) Promoting Sustainable Agriculture and Rural Development. In: *Heart Summit Agenda 21*. United Nations Conference on Environment and Development, Rio de Janeiro, Brazil, Chapter 14. Available at: http://www.un.org/esa/dsd/agenda21/res\_agenda21\_00.shtml.

Van Huylenbroeck G., Vanslembrouck I., Calus M. and Van de Velde L. (2006) Synergies between farming and Rural Tourism: Evidence from Flanders. *Eurochoices* 5(1), 14–21.

Vanselembrouck I., Van Huylenbroeck G. and Van Meensel J. (2005) Impact of Agriculture on Rural Tourism. A Hedonic Pricing Approach. *Journal of Agricultural Economics* 56(1), 17–30.

Vermont S. (2005) National report of Switzerland on environmental services and financing the protection and sustainable use of water related ecosystems. In: *Seminar on Environmental Services and Financing for the protection and Sustainable Use of Ecosystems*, Geneva, Switzerland.

http://portals.wi.wur.nl/msp.

http://ec.europa.eu/europeaid/promotion/casestudies/article\_1837eu\_htm

http://ec.europa.eu/agriculture

http://ec.europa.eu/environment

http://ec.europa.eu/environment/life

http://www.organic-world.net/statistics-eurostat.html

### Session 5.1.

# Effects of political driving forces on grassland management

### Identifying ways to improve competiveness for small-scale livestock farmers in the countries of Eastern Europe

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#### Abstract

Political change in Eastern Europe has led to profound farm structural changes, notably in the newly independent states of the former USSR and Yugoslavia. The focus of this paper is the livestock farming systems of the non-EU East European, western Balkan and Caucasus region. We consider the following: livestock farming in national economies, and recent trends; identification and management of constraints to market access for small-scale farmers and processors; food safety / hygiene and other legislative issues affecting producers and food processors. There are two main sets of issues: developing a trusted and well-regulated marketing system, and overcoming the barriers that prevent small-scale farmers from developing sustainable farming practices. These include access to finance (high rates of interest demanded – often exorbitant – and the short-term nature of most loans), advisory support, technical inputs and veterinary services, as well as harmonization of food safety, traceability, animal recording and veterinary standards towards those of the EU. There is a further need for improving cooperative arrangements, trusted by farmers themselves, for instance through equipment sharing, joint sales/purchases, and development of young farmers clubs, demonstration farms and discussion groups.

#### Introduction

The changing political economy of Eastern Europe since the 1990s has led to profound structural changes in the livestock farming sector, notably in many of the newly independent states of the former USSR and Yugoslavia. The break-up of previously state-run farms has led to the emergence of large numbers of small-scale family farms, often poorly equipped and of insufficient land area for an economically viable business. For small-scale farmers the only way to obtain an adequate income from their farm is through raising production. In this environment, small-scale producers face many obstacles and challenges, notably access to markets and financial services, as well as environmental problems, the need for animal health and livestock improvement, and access to technical knowledge.

The focus of this paper is the livestock farming systems of the non-EU East European, western Balkan and Caucasus region (including Albania, Armenia, Belarus, Bosnia Hertzegovina [BiH], Croatia, Georgia, Macedonia FYR, Moldova, Montenegro, Serbia, Ukraine). Based on the outcomes of an FAO workshop (FAO, 2010) the paper considers the following issues: (1) livestock farming in national economies, and their recent trends; (2) identification and management of constraints for market access for small-scale farmers and processors; (3) food safety and food hygiene and other legislative issues affecting producers and food processors. Solutions considered include: product development; agro-tourism; improving rural infrastructure; improving links between farmers and processors and developing livestock markets; investing in appropriate new technologies; improving access to veterinary services; improving agro-policy measures; developing trust between farmers and state agencies; and improved training for farmers.

#### The role of livestock farming in national economies: trends and problems

Agriculture contributes a significant proportion of GDP in many countries in the region (e.g. Albania 17%, Armenia 40%, BiH 11.4%, Ukraine 12.5%) and the livestock sector typically accounts for around 40–60% of agricultural GDP. Proportions of national workforces employed in agriculture are also high (e.g. 18% in Macedonia). Although there are many national differences, some features are consistent among the countries in the region. Most farms are very small (e.g. Albania 1.13 ha with 1.5 LU/farm) often with structural problems including land fragmentation and difficult road access; they are poorly equipped, and the farmers usually have low levels of training. The mean numbers of livestock per farm are low in all countries except Belarus (most farms have fewer than 5 cows). However, small farms have a socio-economic value at the local scale and provide a social buffer in low-income rural communities; nevertheless, many small farmers are going out of business.

*Livestock numbers.* There has been a general trend of a reduction in numbers of ruminant livestock. The decline in livestock numbers is associated with structural changes following the break-up of the former USSR and Yugoslavia. For instance, during 2000–2009 in Ukraine cattle numbers decreased by 50%; in Serbia cattle, sheep and goats decreased by 20%; and in other countries reductions of >10% are widely reported. Some recent (since 2009) reversals to this trend are reported. In contrast to most countries in the region, Belarus has retained its large farms from the USSR period and these supply most of the marketed beef and dairy cow production; during 2000–2009, milk yields and numbers of cattle and beef production increased to levels similar to or above 1990 levels. Access to the Russian market, combined with technical improvements in animal breeding etc. and their implementation in more concentrated systems, have resulted in a different model to that of the region.

The rest of the region contains many different models also – e.g. the existence and widespread nature of small-scale and subsistence farms in Albania, Armenia, Georgia, Moldova, Serbia and Montenegro owes less to the break-up of state-owned farms and more to residual land ownership having remained with families. The degree of agro-nationalization varied throughout the eastern block from blanket to patchy, and dealing with the state-farm legacy has, and continues to be, non-uniform by respective governments.

*Livestock breeding*. At present, per-animal livestock production levels are generally low, often very low compared with EU averages. Low production is partly due to feed resources and other husbandry practices, and also to quality of livestock breeding. Breeding stock could be improved through artificial insemination (AI), but there is a widespread problem of inaccessibility to AI for many small farms. Animal breeding organizations are very important, both in terms of improving animal performance and at the same time conserving genetic resources of local breeds. Conserving the indigenous breeds is recognized as an important objective, and small farms are often very appropriate for maintaining local breeds. This is especially important if their production can also be linked to niche meat and milk products linked to those breeds, places and production systems (FAO, 2009).

*Agricultural practices*. In addition to livestock breeding constraints, the availability of new technologies and know-how to enable producers to raise their output and efficiency remains low. Conserved feed is generally of inadequate quality and quantity for good animal performance. Pastures in hill and mountain areas are often unused or underutilized, or degraded through poor management. There is widespread lack of knowledge among farmers about nutrient requirements and manure management.

#### Market access

The arrangements for producers to market their livestock on satisfactory terms (contracts with large buyers such as meat processing companies or supermarkets) are generally not well developed, but good examples do show what can be achieved and the problems that need to be addressed in setting up arrangements such as co-operatives, wool and livestock marketing procedures, and developing farmer-led organizations such as young farmers clubs and farm unions. The empowerment of farmers is seen as very important in linking young rural people, especially with their counterparts elsewhere in Europe. Unwillingness to join co-operatives is influenced by past experiences of Soviet-style models of farm cooperatives. Appropriate models of co-operatives need to be developed for small-scale farmers, e.g. a well-positioned regional market that can service several municipalities. Issues of food safety remain a barrier to market access. There is a need to promote EU regulations on animal welfare and good agricultural and good veterinary practices, although increased regulatory requirements are likely to increase the costs to producers. Several countries have introduced animal registration schemes though not all are compatible with EU regulations. Disease control remains an important issue, and has trans-boundary threats, but many farmers lack access to veterinary services. There are also problems due to insufficient suitable abattoirs. Support to protect national cuisines, traditional methods of food preparation and relevant areas of cultural identity protection under EU legislation should be encouraged and promoted. *Financial support, marketing and cooperatives,* Farm businesses need to invest to survive. even just to comply with marketing requirements, and need access to credit. Governmental and institutional support exists for large farms because they are better able to meet the global challenges for increased food production. Many small-scale 'householder' farmers supply their own families and local customers through personal contacts and have no means or knowledge to enable them to access commercial markets. The focus therefore needs to be on enabling producers to develop their own arrangements of cooperation or joint working practices. Examples might include setting up central milk collection points, sharing providers of services or of purchased production inputs, through to sharing the costs of a common milking parlour to enable milk to qualify for quality premiums based on improved hygiene.

#### Other long-term goals

There is a need to reverse the trend of the past decade in terms of livestock numbers and thereby improve local self-sufficiency and enable exported production where appropriate, including recognition for premium and niche products. There is a more general need to improve product quality, with product branding (FAO, 2009) as well as diversification – including developed tourist infrastructure that can bring new business opportunities to rural communities. There is a need for stable incomes and rural development, including infrastructure, capacity building and tackling the serious issues of rural emigration. Improved efficiencies in livestock production are needed. These also need to take account of sustainable resource use and environmental conservation requirements.

#### References

FAO (2009) Linking people, places and products: a guide for promoting quality linked to geographical origin and sustainable geographical indications (Emile Vandecandelaere *et al.* eds.) FAO, Rome, 194 pp. FAO (2010) Best practices in cattle, sheep and goat production ensuring market access for small scale farmers for Central and Eastern European Countries (25–28 Oct., 2010, Budapest, Hungary).http://www.fao.org/europe/meetings-and-events-2010/best-practices-in-cattle-sheep-and-goat-production/en/.

#### An interdisciplinary approach to analyse management tools for sustainable agri-environmental livestock practices: application to the Pastoral Value in the French Pyrenees

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#### Abstract

The aim of this paper is to contribute to the analysis of management tools for sustainable agricultural systems based on natural resources management from the perspective of a more relevant designing process. This management is faced with uncertainty linked to the lack of knowledge about the ecological effects of agricultural practices. New management modes, which involve longer term forecasts and, especially, adaptive management that is adjusting decisions in the course of action, lead us to question the nature of the management tools. We analyszed the Pastoral Value tool used to assess the potential of forage resources in livestock farms in France with management science concepts to better understand the relationships between the nature of the tool and practices. Our findings show how the concepts and paradigms building the chosen tool, strongly shapes practices and how it can be adapted by technicians to their situation within the context of the French Central Pyrenees, emphasizing the role of experiential knowledge.

Keywords: pasture management, decision aid tools, managerial philosophy, Pastoral Value, French Pyrenees Mountains

#### Introduction

Evolution in agricultural production modes attempts to reconcile food production, the reproduction of natural resources and the management of natural habitats. It entails a change of management paradigm towards adaptive management to face with an uncertainty linked to the lack of knowledge about ecological systems (Voß et al., 2006) in response to practices. This leads to question the nature of the tools required to accompany stakeholders in management involving adjustments in the course of action. The case of pastoral livestock farming systems is particularly relevant, as designers of tools used to develop more sustainable livestock practices are confronted by the lack of knowledge to predict the evolution of dynamics of natural vegetation (rangelands and grasslands) under the effect of grazing practices. The aim of this paper is to propose a conceptual framework for better analysis of natural resource management tools capable of adapting to changes in livestock practices. We analyse the nature of tools by their cognitive and managerial content, through the use of an interdisciplinary approach at the intersection of agroecology and management sciences. The 'managerial philosophy' defines the system of concepts that denote the objects and objectives constituting the targets of a rationalization (Hatchuel and Weil, 1995); for instance, the optimization of decisions for operational research, the modelling of knowledge for expert systems. We analyse the nature of a currently used tool, pastoral value, focusing on the way that how their cognitive content and their presuppositions tools, provide a framework for action.

#### Methods

Our analysis is based on an actual pasture management situation in the French Central Pyrenees chosen as a typical target of agri-environmental management policies and for which a group of

stakeholders (pastoralists, naturalists, Natura 2000 local coordinator) is questioning the tools to be used for pastoral management. We focus on one of these tools known as the 'agroecological vegetation assessment' (Bornard and Dubost, 1987) on the basis of calculations of the Pastoral Value established by Daget and Poissonet (1971). Our data were taken from long, semi-directive interviews carried out from 2008 to 2010, which focused on the characteristics of this tool, the knowledge it contains and its genealogy (how and by whom it was designed) and its use by the group of stakeholders. The following people were interviewed:

designers of the tool used to carry out pastoral assessments (four interviews). These interviews focused on the origin, design and content of the method, as well as on its diffusion;
end-users of the tool (nine interviews) on their professional careers, their current activity and their practices in relation to the use of the tool.

We completed these interviews with results from scientific articles and technical documents published on the 'Pastoral Value' tool, technical documents issued from the group activity. We also observed seven indoor and field meetings of the group during which the actors discuss and implement the tools that they use to develop vegetation surveys. The data (transcribed interviews, technical documents and scientific articles) were structured into a database using NVivo computer-aided qualitative data analysis software. The analysis was thus carried out in two steps, descriptive coding, close to the data, making it possible to describe and later retrieve information collected, and analytical coding, making it possible to assign conceptual categories to text segments in the corpus. This approach therefore allowed us to jointly analyse a particular tool and use situation while proposing an analytical grid as a generic result of this analysis.

#### **Results and discussion**

In a first step, we built a conceptual framework and an analysis grid to decipher the foundations of agricultural tools crossing three dimensions of management instruments, as developed by Hatchuel and Weil (1995): i) the technical substrate corresponding to the material dimension, ii) the management philosophy dimension integrating the designer's intentions and the targeted objectives, and iii) the organizational model defining users and utilisation modes (Gross *et al.*, 2011). The application of this grid shows that the Pastoral Value is built on phytoecology concepts and methods, which determine strongly the way to define pastoral resource and therefore the way in which management recommendations are drawn up (Table 1). Two main postulates are the qualification of the resource by the nutritional value of each plant species and the optimization of the intake/plant production ratio as the resource valorisation principle.

We explored then how the conceptual foundations of the tool design shape action and lead to difficulties for managers facing to the underestimation of the woody species resource, the lack of control of their dynamics and the local contextualisation of the method. Strategies are therefore developed by actors based on adjustments in estimation of the resource to minimize potential errors of diagnosis. They used the intrinsic flexibility (example PV value intervals by ecofacies) to modify the values obtained as a function of his perception of the situation that he studied. They can also integrate a safety margin by introducing a 'minus factor' to adjust the Recommended Stocking Rate to take in account the constraints of the livestock systems. Besides these changes, they try to build their proper indicators for shrubs management from survey and experimental trials.

#### Conclusions

The conceptual grid proposed by juxtaposing the analysis of tool content with its use within action allows analysing the relevance of tools. This cross-analysis applied to Pastoral Value makes it possible to describe ways to adapt tools to a given situation and, in particular, the

Grid elements		Application to the Pastoral Value				
Technical	Object measured	Ecofacies				
substrate	Measurement and	- Zoning of the pastoral unit into ecofacies				
	calculation pro-	- For each ecofacies:				
	cedure	1. Determining the Specific Frequency (SFi) of a species by botanical				
		surveys				
		2. Calculation of the Specific Contribution (SCi): SCi = (SFi $\Sigma$ SFi) $\times$ 100				
		3. Calculation of the PV: $PV = 0.2 \times \Sigma(SCi \times SFi)$				
		3. Calculation of the Theoretical Forage Potential: TFP = $PV \times k$				
		4. Recommended Stocking Rate: RSR = TFP × minus factor				
Management	Management ob-	Management object: the ecofacies.				
philosophy	jectives and target	Aim: to establish a grazing plan that makes it possible to optimize the pasto-				
		ral valorization of the grassland resource.				
	Conceptual basis	Basis and principles of phytoecology.				
	and management	Vegetation value as the addition of intrinsic plant species value.				
	object	Physiological approach of animal science.				
	Interpretation rules,	Woody species have an SFQ $i = 0$ and therefore have no food value.				
	postulates and ef-	The food value of a species is assessed on the basis of its intrinsic qualities				
	ficiency criteria	and can therefore be transposed from one location to another.				
		Optimizing resource intake means consuming the maximum biomass at the				
		full-growth phenological stage of the grass (not at more advanced stages				
		of maturation).				
		There is a proportional relationship between the PV and the Recommended				
		Stocking Rate: the higher the PV, the greater the RSR.				
Organization-	Targeted end users	In the beginning, scientists including designers, followed by pastoral techni-				
al model		cians.				
	Ideal use situations	Not described explicitly, but:				
		- Field work to understand the pastoral system and qualify the vegetation of				
		the area;				
		– Interactions with farmers and shepherds.				

Table 1. Management tool analysis grid and its application to the Pastoral Value

role of experiential knowledge (Kolb, 1984). Designing tools which are more adapted to the management in uncertainty makes it necessary to mobilize knowledge acquired by the stakeholders during their management experiences and gives rise, as a result, to the question of the articulation of scientific and experiential knowledge in tools design. Finally, it entails a major change in scientific knowledge production, requiring a shift from descriptive to more engineering-oriented sciences aimed at producing actionable knowledge (Argyris, 1994).

#### References

Argyris C. (1994) Knowledge for Action. Jossey-Bass, San Francisco.

Bazeley P. (2007) NVivo computer-aided qualitative data analysis software Qualitative data analysis with NVivo, Sage (London).

Bornard A. and Dubost M. (1987) *Exploitation de pelouses et landes subalpines par des bovins et des ovins*. Ed. CEMAGREF, Compte-rendu de recherche 211–245.

Daget P. and Poissonnet J. (1971) Une méthode d'analyse phytoécologique des prairies. *Annales Agronomiques* 22, 5–41. Gross H., Girard N. and Magda D. (2011) Analysing theory and use of management tools for sustainable agrienvironmental livestock practices: the case of Pastoral Value in the French Pyrenees. *Journal of Sustainable Agriculture* 35, 5, 550–573.

Hatchuel A. and Weil B. (1995) *Experts in Organisations. A Knowledge-based Perspective on Organizational Change*. Translation Walter de Gruyter. Berlin-New-York.

Kolb D.A. (1984) *Experiential learning: experience as a source of learning and development*. Prentice Hall, Englewood Cliffs, NJ, 38 pp.

Voß J.-P., Newig J., Kastens B, Monstadt J. and Nölting B. (2006) Steering for Sustainable Development – A typology of empirical contexts and theories based on ambivalence, uncertainty and distributed power. *Journal of Environment Policy and Planing* 9, 3/4, 193–212.

#### DAIRYMAN for a more efficient use of resources by dairy farms

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#### Abstract

Inputs of feeds and fertilisers in dairy cattle systems are high because high quality feeding has to be secured. Unfortunately, the efficiency of utilisation of these resources and of feeds and manures produced by the farm itself is much lower than is supposed to be attainable. Improved resource management will reduce inputs and result in lower costs of purchases and reducedenvironmental pollution. In the DAIRYMAN-project, ten dairy regions in NW Europe cooperate in improving resource management of dairy farms. The INTERREG NWE project was initiated by the EGF Working group 'Dairy farming systems and Environment' and started in November 2009 for a period of four years. About 130 commercial pilot farms and 9 Knowledge Transfer Centres participate. Management tools in use by farmers are exchanged and innovations are tested at a farm scale. Standards are made concerning how to estimate farm performances (losses of noxious N-, P- an C-compounds; economics) and how to set up a farm development plan that meets both economic and environmental go-als. The paper describes the project (http://www.interregdairyman.eu) and its value for non-participating regions and for the EGF community.

Keywords: dairy farming system, environment, resource utilisation, EGF

#### Introduction

Dairy farming is a major economic activity in Northwest Europe. Soil and climate conditions are suitable, the infrastructure is excellent and there is a large market for dairy products. However, environmental sustainability in most dairy regions is low. This is mainly due to a poor efficiency of the utilisation of the resources of fertilizers and feeds. Inputs of these products are high, compared to inputs of beef cattle systems, because high quality feeding has to be secured. Poor utilisation of feeds and fertilisers leads to high levels of harmful emissions of nitrogen, phosphorus and carbon compounds. This inhibits the ability of rural areas in these mostly densely populated regions to deliver other valuable services apart from food production, such as clean water or opportunities for recreation. Moreover, the poor utilisation of increasingly expensive resources threatens the economic viability of dairy farms.

From experiments and farm analyses we know that utilisation of resources can be greatly improved, and in a financially profitable way, by reconsidering farm management. Therefore, during its pre-conference workshop in Gent (2007), the EGF Working group 'Dairy farming systems and Environment' initiated the INTERREG NWE project DAIRYMAN. The project in which 14 partners cooperate started in November 2009, for a period of 4 years. The main target is to strengthen rural communities in regions where dairy farming is a main economic activity and a vital form of land use, by improving the resource management of commercial dairy farms. It will lead to a more competitive dairy sector, stronger regional economies and an improved ecological performance within the rural area.

#### The Ten participating regions

The DAIRYMAN project had to be restricted to the territory of INTERREG NWE. The participating regions are Brittany (FB), Pays de la Loire (FL), Nord Pas de Calais (FC), the

Southwest of Ireland (IR), Northern Ireland (NI), Flanders (BF), Wallonia (BW), Baden-Württemberg (DW) Luxembourg (LU) and the Netherlands (NL). There is cooperation with the Po-valley (IT) by an EU-LIFE funded project.

For each region sustainability was described at 3 levels (whole region, agriculture within the region, dairy farming within agriculture), using an agreed list of indicators for economic, ecological and social aspects. A report describing the process of implementing EU environmental directives (who did what) and the results (legislation) was made for each participating region. Reports are already, or soon will be, available on the project website (http://www.interregdairyman.eu). In this way, sustainability topics were made clear and could play a role in dialogues with farmers and other stakeholders about the needs for farm improvement.

#### The 131 participating pilot farms

A network of 131 progressive commercial dairy pilot farms span the participating regions and serve as focal points to inspire local farmers. These pilot farms are assisted in adopting improved management strategies, by farm advisers and scientists. For each farm, a development plan was made in a standardised way, guiding the farmer to the future and facilitating effective communication between pilot farmers regarding their farm strategies.

The impact of new working methods on the sustainability of the pilot farm is monitored and evaluated. Experiences will be used to write a manual on how to assess environmental and economic farm performances and to set up a farm development plan, to be used by farmers, farm advisers and agricultural students.

Several management tools are in use by farmers in the participating regions in an attempt to utilise more accurately their fertilizers and feeds. However, these tools are often unknown in other regions or not useful because they have not been adjusted to the regional settings. An inventory resulted in 65 tools that now are available on the internet with an indication of usefulness in other regions and a contact address for assistance.

During the project period, pilot farmers will visit their colleagues in other regions twice, to learn about diversity in European dairy farming (Table 1) and its backgrounds, to exchange experiences and to be inspired for further farm improvements.

#### The Nine participating Knowledge Transfer Centres

Nine Knowledge Transfer Centres are situated in the DAIRYMAN regions to explore innovative farming systems, to test innovations and to disperse knowledge. By cooperation these tasks can be done more efficiently. The participating Centres are Trévarez (FB), Derval (FL), De Marke (NL), Moorepark/Solohead (IR), Hillsborough (NI), Hooibeekhoeve (BF), Gembloux (BW), Bildungs- und Wissenszentrum für Viehhaltung, Gruenlandwirtschaft, Wild und Fischerei (DW) and Lycée Technique Agricole (LU).

#### The value of DAIRYMAN for European dairy farming and for the EGF community

DAIRYMAN is not only profitable for the participating regions. Inventoried management tools and standards to calculate environmental and economic performances of dairy farms are freely available for all. There will be a manual for the set-up of a farm improvement plan. DAIRYMAN is also important for the image of dairy farming in Europe. Recently, the EC agriculture commissioner Ciolos used one of the pilot farms as the platform to present his proposals for a reform of Common Agricultural Policies after 2013, demonstrating that these farms are symbolic for the future.

1 1 0 0	· ·									
Region	LU	NL	BF	BW	DW	FC	FB	FL	IR	NI
Land use (ha)										
Grass	76	46	31	56	66	50	45	55	67	92
Fodder crops	36	9	21	14	65	28	30	40	2	12
Arable crops	13	0	5	25	8	37	27	58	0	0
Milk production (tonne)										
Farm quota	462	1017	687	482	806	538	405	536	498	1228
Milk ha <sup>-1</sup>	3.7	19.4	12.6	5.8	6.6	5.2	4.3	4.0	7.6	11.5
Milk cow <sup>-1</sup>	7.6	8.5	8.1	7.4	8.3	8.0	6.4	8.0	5.3	8.1
Cattle (no.)										
Milking cows	59	120	85	65	97	69	64	68	96	150
Young stock cow <sup>-1</sup>	1.2	0.7	0.8	0.9	1.1	1.1	0.8	1.0	1.0	0.7
Beef cattle	55	0	2	31	3	42	19	22	0	0

Table 1. Diversity in dairy farming: average data of the pilot farms in 2009 (for codes of regions see 'The 10 participating regions')

For the EGF community it is important to learn that EGF-Working Groups can initiate projects like DAIRYMAN in which its members truly cooperate. On the other hand, projects like DAIRYMAN can facilitate the preparation of workshops of the EGF-Working Groups. These workshops provide opportunities to exchange thoughts and experiences. In that way they strengthen the personal network and professional functioning of EGF-members. In addition, workshops can lead to further collaboration in projects like DAIRYMAN.

#### Experiences with ecosystem services on farms: a pilot study towards CAP reform

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#### Abstract

A reward system for ecosystem services was developed for a group of farmers in Winterswijk (NL). Most of these services are connected to multifunctional land-use activities. A farmer erans points for a range of activities, which are valued with more or fewer points depending on their importance for landscape and/or community activities. Total payment is based on sum of all points multiplied by a payment per point. The local community has adopted this payment system, and in December 2008 launched a countryside fund to reward farmers for offering green-blue services. Since spring 2011 the region is also a pilot area to study options for the reform of the European Common Agricultural Policy (CAP) and 97 farmers participate in this pilot study. From 2013 onwards the CAP will focus on offering more services to the public, rather than just food production. Maintaining landscapes, employment, environment, climate change and biodiversity is a great and new challenge for farmers of Winterswijk will build up experience with the provision of these services. The Dutch Government will use the results of the pilot in the negotiations with the European Commission.

Keywords: ecosystem services, multifunctional grasslands, landscape conservation, European Common Agricultural Policy, regional development

#### Introduction

Multifunctional land use is an option to increase economic and environmental sustainability of farms, and it makes a region more attractive for local inhabitants and visitors (Wiggering et al., 2006). Between 2002 and 2004, a group of 14 farms was studied in the Winterswijk region (eastern part of the Netherlands). The area is a small-scale landscape with high nature and landscape values, consisting of a mosaic of grasslands, arable fields, hedgerows and woodlots. The farms differed in their activity, and comprised dairy-, beef-, young stock rearing-, arable-, pig- and mixed-farming. Four were organic farms, and they differ in their level of function combinations. The results indicated that a combination of agronomic, ecological and environmental goals is possible, and that there are possibilities to combine high biodiversity with a relatively high level of production (Korevaar and Geerts, 2007). In most cases, multifunctionality is not profitable for the individual farmer. However, for the region as a whole it offers good opportunities to create extra income from recreation and tourism. Financial deals between farmers and local community and tourism sectors are necessary to reallocate this extra income and to achieve a more balanced division of costs and revenues. A rewards system for ecosystem services was developed and tested in 2007 and 2008 at the request of the same group of farmers. Experiences are presented and discussed in this paper. A driving force behind regional development in this area is the foundation WCL Winterswijk; it is a platform in which municipality, farmers' organization, owners of small estates, local nature and environmental groups, recreation and tourism sector, local industries and citizens groups of the different villages cooperate. WCL Winterswijk aims to maintain the beautiful small-scale landscape, develop the agricultural infrastructure and improve the ecological values of the region. Farmers play an important role in the maintenance of the landscape, and continuation of farming is therefore essential for landscape conservation.

#### **Rewarding ecosystem services**

Activities or ecosystem services (De Groot *et al.*, 2002) that should be rewarded are chosen at a local level, including a number of features that are typical for that region, like restoration of old arable fields and adjacent (steep) edges. The activities are evaluated with points, depending on their importance for landscape and/or community, and the acreage or intensity of that activity. Scores are multiplied by a payment per point, which results in the total payment to the farmer. The incentive is that farmers are rewarded for their effort instead of compensating them for production losses, which is the case in most agri-environmental schemes. WCL Winterswijk adopted this payment system and launched a countryside fund to reward farmers for offering ecosystem services in December 2008. It was agreed that in the first years 75% of the budget should be supplied by the province and 25% by local and private funds; later on it would be 50–50%. Until now the success of this countryside fund is limited because the province is still debating about the terms under which the provincial budget could become available. In 2009 and 2010 the same group of farmers continued with offering green-blue services and got some payments from the countryside fund, but there were no funds to enlarge this group or to make long-term agreements.

WCL Winterswijk was disappointed about the lack of progress, but found another way to stimulate farmers to broaden their farming system. The region applied to become a CAP-pilot area to study options for farmers in offering services to the community. One of the aims of the European Common Agricultural Policy (CAP) after the reform in 2013 is to make the policy fairer, greener, more efficient, more effective and more understandable, so that it offers more services to the public than just food production (EC, 2011). The Dutch Government is interested in the possibilities of local collectives, like WCL Winterswijk, to offer high quality services in an efficient and cheaper way than the top-down organization of the present CAP payments. Since spring 2011, a total of 97 farmers have participated in the pilot study.

#### **Results and discussion**

Land use in Winterswijk is dominated by grassland (65%) and maize silage (24%). Other crops are cereals (4%) and potatoes (6%). Only 1% is used for horticulture, tree nurseries and fruit production. Ninety-seven farmers participate in the CAP-pilot, that is 30% of all farmers in the region. Forty-five of the participants are specialised dairy farms, 7 beef cattle, 3 pigs, 3 poultry and 3 are arable farms. Nineteen are 'mixed farms', 3 are small estates and 15 have another farm type. The average farm size of the participants is 33 ha, which is a significantly larger area than the 23.5 ha average farm size in the region. Table 1 shows the number of farms that deliver a certain ecosystem service. The preservation of small fields and old meadows is one of the most popular services (Table 1) which contribute highly to the maintenance of the typical small-scale landscape of Winterswijk. Rewarding farmers for maintaining these small fields fits into the new CAP policy; otherwise, farmers would enlarge their fields for greater efficiency (Rienks, 2008). In the pilot study, the farmers of Winterswijk build up experience on the provision of services to the local community. Feedback from the farmers, WCL Winterswijk and the Ministry of Economic Affairs, Agriculture and Innovation, is positive about the first results of the CAP-pilot study. There was a high willingness among farmers to participate. Taking care of landscapes, employment, environment, climate change and biodiversity is a great and new challenge for most farmers. The project is inspiring. The results of the pilot study will be used by the Dutch government in the negotiations towards a new European Common Agricultural Policy from 2013 onwards. The region is waiting for the outcome of the further CAP negotiations.

Compiles (activity)	Number	Unito	Area (ha)	Payment (€) per	Total costs
Service/activity	of farms	Units	or length (km)	unit	(1000 €)
Biodiversity					
	07			50 field 2–3 ha	20.3
Procorruption of small fields	(-011)	ha	045.0	125 field 1-2 ha	49.0
rieservation of small fields	(- all	IIa	945.0	250 field 0.5-1 ha	28.8
	Tarms)			400 field <0.5 ha	12.7
Cleaning grassy field margins	76	km	101.6	500	50.8
along forests and hedgerows	70	KIII	101.0	500	50.8
Reintroduction of cereals	19	ha	31.4	500	15.7
Sowing arable field boundary	12	ha	5 5	2 000	11.0
species	12	IIa	5.5	2,000	11.0
Unharvest cereal crop	4	ha	1.8	1,400	2.6
Overwinter stubbles	7	ha	10.1	250	2.5
Reintroduction of species-rich	7	ha	0.4	1 400	12.2
grasslands	/	па	9.4	1,400	15.2
Preservation of old meadows	62	ha	538.5	50	26.9
Sowing species rich margins along	2	ha	0.5	1 500	0.8
grasslands	Z	па	0.5	1,500	0.8
Introduction of grass-clover swards	15	ha	36.4	250	9.1
Landscape					
Maintenance of woodlots $< 0.5$ ha	28	ha	7.3	5,000	36.3
Maintenance of solitary trees	28	number	110	50	5.5
Fencing solitary trees	14	number	47	100	4.7
Conservation of steep margins	21	1	1.0	5 000	0.0
along arable fields	21	па	1.8	3,000	9.0
Maintenance of sheltered fruit trees	21	number	315	20	6.3
Water quality					
Introduction of catch crops	21	ha	51.3	250	12.8
Education and open farms					
Education and farms open to visi-	22	hours	170	50	05
tors	22	nours	170	30	8.5
Footpaths over farm land	8	km	7.3	500	3.6
Total costs					330.1

Table 1. Green-blue services on the farms participating in the CAP-pilot in Winterswijk in 2011 (preliminary results)

#### References

EC (2011) Cap Reform – an explanation of the main elements. Memo/11/685. 12 October 2011. European Commission, Brussels.

De Groot R.S., Wilson M.A. and Boumans R.M.J. (2002) A typology for the classification, description and valuation of ecosystem functions, goods and services. *Ecological Economics* 41, 393–408.

Korevaar H. and Geerts R.H.E.M. (2007) Productivity, biodiversity and nitrate in groundwater of multifunctional grasslands. In: *High Value Grassland. Providing biodiversity, a clean environment and premium products.* British Grassland Soc., Occasional Symposium 38 Cirencester, UK, pp. 64–69.

Rienks W. (2008) Large scale dairy farming in small scale landscapes. Conference '*Transitions towards sustainable agriculture, food chains and peri-urban areas*'. Wageningen, 26–29 October, 2008. www. agricultureintransition.wur.nl/UK/Abstracts/

Wiggering H., Dalchow C., Glemnitz M., Helming K., Müller K., Schultz A., Stachow U. and Zander P. (2006) Indicators for multifunctional land use. Linking socio-economic requirements with landscape potentials. *Ecological Indicators* 6, 238–249.

## Migratory geese foraging on grassland: Case study in the region of Flanders (Belgium)

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#### Abstract

Every winter nearly 100 000 migratory geese visit North-western Flanders (Belgium), including several protected species such as the pink-footed goose (*Anser brachyrhynchus*). The geese mainly forage on agricultural grassland, where they remove all the green parts and leave substantial amounts of droppings. In 2009 several farmers' concerns about this phenomenon were thoroughly investigated. The main findings revealed that grass production on grazed parcels is reduced by 450 kg DM ha<sup>-1</sup> on average at the time of the first cut around 1 May. On the same parcels, soil nitrogen addition from goose droppings did not far exceed 10 kg ha<sup>-1</sup>, a small amount in comparison to the farmers' average annual fertilization rate. No negative effect on grass fodder quality was found; even a small but significant increase in crude protein content was observed as well as a decrease in crude fibre content. The results of this study laid the foundation for measuring grass yield losses due to grazing by protected wildlife species, now used in a compensation scheme for farmers.

Keywords: migratory geese, wildlife damage, grass yield, fodder quality, nitrogen addition

#### Introduction

Every winter, several geese species migrate from their Arctic environment, e.g. in Siberia or Svalbard, to western Europe. This includes the coastal regions of North-western Flanders, where they were first observed in 1958 (Kuijken *et al.*, 2005). Since the mid-1990s they have been counted systematically, twice a month, during winter (Devos *et al.*, 2005). The species observed in the largest numbers are the greater white-fronted goose (*Anser albifrons*) and the pink-footed goose (*Anser brachyrhynchus*), followed by the greylag goose (*Anser anser*). The actual number of migratory geese is hard to assess as the species arrive and leave at different times, but maxima of simultaneous counts over all species are traditionally observed in the months of December and January, reaching about 90 000 individuals in the years between 2001 and 2004 (Devos *et al.*, 2005).

Although their presence is enjoyed by many, local farmers can suffer from crop damage caused by large geese numbers foraging on their fields (Van Gils *et al.*, 2009). In 2008, the Flemish government started the development of a compensation scheme for yield losses. However, measurement tools and protocols for damage assessment were largely unavailable. This was especially the case for grasslands, the primary goose habitat (Devos *et al.*, 2005).

#### Materials and methods

A selection of five agricultural grassland parcels was made, each with a history of being grazed by migratory geese in winter. Four fenced exclosures of 3 m by 6 m on each parcel made sure that ungrazed reference plots remained available, with grazed plots of the same dimensions at 1 m distance. Within the latter, counts of geese droppings were performed every 10 days to assess grazing intensity as expressed in 'goose-days' per ha. One goose-day (24 hrs) on grassland accounts for 125 droppings. Additionally, dropping counts were performed on transects crossing the whole parcel, counting within a square frame  $(0.25 \text{ m}^2)$  placed on the ground every 25 m.

Grass yield losses (dry weight) were assessed in spring by harvesting and comparing the grazed versus ungrazed plots. Grass fodder quality parameters – crude protein and crude fibre content – were assessed with NIRS (Near-Infrared Spectroscopy, Shenk *et al.*, 1992).

To assess a possible fertilizing effect of goose droppings, calculations of theoretical nitrogen (N) addition were made, based on chemical analysis of droppings (Groot Bruinderink, 1987) combined with dropping counts.

#### Results

Grazing intensity of the experimental plots varied greatly, from 112 up to 2 944 goosedays per hectare (Table 1). Calculations based on transect counts of droppings were higher compared to those based on plot counts. Grass yield losses at the first cut in spring averaged 450 kg DM ha<sup>-1</sup>. Grass fodder quality was affected by grazing in winter. Average crude protein content increased from 20.2% in ungrazed plots to 21.2% for grazed plots (P = 0.027). The opposite trend was observed in crude fibre content, which decreased from 23.5% in ungrazed to 22.6% in grazed plots (P = 0.009). Calculations based on transect dropping counts showed that nitrogen additions between 3.6 kg ha<sup>-1</sup> N and 13.7 kg ha<sup>-1</sup> N could be expected (Table 1).

Field and	GDplot	GDtransect	Yield loss	DCP	DCF	N add
Field code	(# ha <sup>-1</sup> )	(# ha <sup>-1</sup> )	(kg ha <sup>-1</sup> DM)	(%)	(%)	(kg ha <sup>-1</sup> N)
Klem03	1 458	2 747	494	+0.3	0.0	12.6
Klem04	2 219	2 944	411	+0.2	-0.5	13.7
Klem05	589	1 248	420	-0.1	-0.2	5.9
Asse03	661	1 422	410	+2.7	-2.2	6.7
Hoek02	112	475	538	+1.8	-1.5	3.6

Table 1. Overview of data from five grasslands - mean values of four plots

GDplot: total of goose-days per hectare, based on dropping counts in grazed plots.

GDtransect: total of goose-days per hectare, based on dropping counts on transects.

DCP: difference in crude protein content (grazed - ungrazed).

DCF: difference in crude fibre content (grazed - ungrazed).

N add: theoretical nitrogen addition, calculations based on GDtransect.

#### Discussion

To assess grazing intensity, transect counts of droppings are probably more accurate than plot counts, as the first are spread over the parcel, therefore including more spatial variability. Moreover, in this experiment a potential deterring effect from the exclosures in the immediate vicinity (1 m) could be expected in the grazed plots.

Farmers' concerns about yield losses due to winter grazing by migratory geese proved to be correct, with an average loss of 450 kg ha<sup>-1</sup> DM. Differences in grazing intensity were much greater than differences in the resulting grass yield losses at the first cut in spring. This could be partially explained by goose behaviour and grassland management: grassland is cut to create a short sward before winter (5 to 8 cm). The geese can only remove a certain amount of foliage, as they can graze the sward to about 2 cm. However, lack of food does not prevent geese from using the terrain as a roosting site, especially overnight. Doing so, they leave significant amounts of droppings, thereby creating a misconception about the term 'grazing intensity' as determined by dropping counts.

Moreover, grass yield losses can be greater in the case of wet soil conditions during grazing, with geese trampling and thus sealing the soil, especially on autumn-sown grassland. This was reported by farmers but was not noticed during this one-year experiment.

Grass fodder quality was affected slightly positively. Swards that are grazed in winter probably have a higher proportion of fresh sprouted foliage in spring. A stronger effect was noticed on parcels that were less intensively managed (Asse03 and Hoek02).

Nitrogen addition due to geese droppings is low compared to the annual input of nitrogen fertilizer in common farming conditions in Flanders: a maximum of 245 kg N available per ha for a management system of grazing combined with cutting.

#### Conclusions

Migratory geese foraging on grassland in winter are able to cause yield losses at the first cut in spring, and in this study it was estimated to be about 450 kg ha<sup>-1</sup> DM on average. Fodder quality of the sward can be affected in a positive way, with a small increase in crude protein content and a small decrease in crude fibre content. Nitrogen addition by goose droppings is negligible compared to common agricultural practices of fertilizing in the region of Flanders. This study provides useful information for measuring grass yield losses due to grazing by protected wildlife species, which can be used in a compensation scheme for farmers.

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#### References

Devos K., Kuijken E., Verscheure C., Meire P., Benoy L., De Smet W. and Gabriëls J. (2005) Overwinterende wilde ganzen in Vlaanderen, 1990/91 – 2003/04. Natuur.oriolus 71 (Bijlage), 3–20.

Groot Bruinderink G.W.T.A. (1987) Wilde ganzen en cultuurgrasland in Nederland. CABO publicatie, 422. Centrum voor Agrobiologisch Onderzoek (CABO), Wageningen. 206 p.

Kuijken E., Verscheure C. and Meire P. (2005) Ganzen in de Oostkustpolders: 45 jaar evolutie van aantallen en verspreiding. *Natuur. Oriolus* 71 (Bijlage), 21–42.

Shenk J.S., Workman J.J. and Westerhaus M.O. (1992) Application of NIR spectroscopy to agricultural products. In: Burns D.A. and Ciurczak E.W. (eds). *Handbook of Near-Infrared Analysis*, pp. 383–431.

Van Gils B., Huysentruyt F., Casaer J., Devos K., De Vliegher A. and Carlier L. (2009) Project winterganzen 2008–2009: onderzoek naar objectieve schadebepaling. INBO.R.2009.56. 87 p.

## Stakeholders' requirements and expectations with respect to multi-functionality of grasslands in Europe

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#### Abstract

The European project Multisward aims to increase reliance on grasslands and on multi-species swards for competitive and sustainable ruminant production systems. This contributes to food security and enhanced environmental goods. As part of Multisward, an inventory was made of requirements and expectations of stakeholders with respect to the multi-functionality of grasslands in EU countries. The traditional foursome of primary producer, policy maker, research and advice were identified as the most important stakeholders, followed by non-governmental organisations (NGOs) (nature, environment), education and industry. A first international stakeholder consultation revealed the appreciation of current and future functions of grasslands in Europe. In general the economic functions of grasslands, mainly feed for herbivores, were considered the most important. Stakeholders expected this to remain unchanged in future. Environmental functions of grasslands were placed second: water quality and quantity, adaptation to climate change, mitigation and biodiversity. Finally, social services were mentioned.

Keywords: economic function, grassland, multi-functionality, stakeholders

#### Introduction

During the last 40 years the European grassland area has significantly reduced, by 15 M ha in favour of the production of fodder maize and other annual crops (FAOSTAT, 2011). Even marginal grasslands tend to be abandoned, particularly in mountainous and Mediterranean areas, where they are of crucial importance for preserving biodiversity, protecting soils against erosion and maintaining the local population density.

The increasing global demand for meat and milk, environmental concerns about the sustainability of intensive production systems and concerns about food quality and safety favour an increasing role for grassland-based ruminant production systems in the future. These systems are likely to be more environmentally sound, economically viable, productive and efficient with respect to scarce inputs. Grasslands, with their multifunctional roles, provide a good basis for developing more sustainable production systems in the long term (Peyraud *et al.*, 2010). The FP7-funded European project Multisward (www.multisward.eu) aims to increase reliance on grasslands and on multi-species swards for increased sustainability and competitiveness of ruminant production systems, increased food security and enhanced environmental goods. In this project, stakeholder participation is being used to improve the scope for anchoring new developments in grasslands use, creating a joint learning environment and ensuring that the developments are based on a better understanding of the perspective of stakeholders on grasslands (Woodhill *et al.*, 2009). Thus far, the requirements and expectations of stakeholders with respect to multi-functionality of grasslands in Europe are not fully known. The aim of this study, which is part of the Multisward project, is to determine these requirements and expectations.

#### Materials and methods

Stakeholders' requirements and expectations were studied by an international team of researchers from the Netherlands, Ireland, France, Poland and Italy. Firstly, a stakeholder analysis was undertaken to identify the people or institutions having a clear stake in the multi-functional use of grasslands, or being in the position to play an important role in the development and implementation of newly found management options for multi-species swards. Results have been obtained from nine countries throughout Europe: Norway, Ireland, United Kingdom, the Netherlands, Belgium, France, Switzerland, Poland and Italy.

Secondly, 25 representatives of important stakeholder groups across Europe, as defined in the stakeholder analysis, were individually invited to an international stakeholder meeting with an interactive program. They represented the most important grassland stakeholders in Europe. The aim of the meeting was to determine the appreciation of current and future functions of grasslands in Europe. This was achieved using workshops in small groups and general discussions summarising the results. The meeting followed the 23th General Meeting of the European Grassland Federation in Kiel in 2010.

#### **Results and discussion**

The stakeholder analysis revealed that the most important stakeholders were the traditional foursome of primary producer, policy maker, research and advice (Pinxterhuis, 2011). Following these most important stakeholders, there were stakeholders of intermediate importance, including nature conservation non-governmental organisations (NGOs) and for environmental protection NGOs, processing and seed industries, education and consumers. The stakeholders identified as being of lesser importance included tourism, recreation and animal welfare NGOs, feed, fertilizer and machinery industries, media, retail and water agencies.

The attendants of the first international stakeholder meeting came from six stakeholder groups (advice, education, farming, industry, policy and research) and five European regions (Atlantic, Continental, Mediterranean, Mountainous and Nordic).

The international stakeholder consultation revealed the appreciation of the current and future functions of grasslands in Europe (Pinxterhuis, 2011). In general the economic functions of grasslands, mainly feed for herbivores, were considered the most important. It was expected that this will remain unchanged in the future. The economic functions entail providing feed for herbivores for the production of meat, milk or wool, and feed for bees for honey production. Next to this, there are more recent economic functions such as providing plant fibre, raw material for bio-energy production, or offering attractive landscapes for tourism or recreation. In some cases participants linked the economic functions to aspects such as 'providing labour', 'keeping people in rural areas', 'maintaining standard of living', all referring to maintaining populations in marginal areas.

Environmental functions of grasslands were generally placed second. Water quality and quantity were mentioned first. How water quantity is interpreted depends on region: in some regions sufficient supply and protection of soil from erosion is important, while in other regions providing flood plains is relevant. In general, grasslands were seen as a potential tool to adapt to climate change. Mitigation of climate change was also frequently mentioned: permanent grasslands provide carbon sequestration, although forestry was seen as a strong competitor for this function. The third environmental function mentioned was biodiversity. In many European areas, grasslands provide unique ecosystems. Preserving these aids the global challenge to maintain biodiversity.

The social services of grasslands were also recognized. Apart from providing livelihood (lifestyle, labour, income; mentioned under economic functions), grasslands and their management are often part of the local culture. Amenities such as landscape for tourism and space for recreation and sports were also mentioned.

Even though only a relatively small group of stakeholders attended the meeting, the obtained results are valuable, since they represent the opinion of important stakeholders throughout Europe. Furthermore, results have been used to develop a questionnaire, which is currently open for responses at www.multisward.eu. Also, national stakeholder meetings have been and will be held in 2011 and 2012 in the Netherlands, Ireland, France, Poland and Italy. Detailed results of these meetings and the questionnaire will become available in 2013.

#### Conclusions

International stakeholders with a clear stake in the multi-functional use of grasslands considered the economic functions of grasslands, mainly feed for herbivores, as the most important functions of grassland in Europe. They expected this to remain unchanged in the future. Environmental functions of grasslands such as water quality and quantity, adaptation to climate change, mitigation and biodiversity were placed second. Finally, social services were mentioned.

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#### References

FAOSTAT (2011) http://faostat.fao.org/. Food and Agriculture Organization of the United Nations, Rome, Italy. Peyraud J.L., Van den Pol-van Dasselaar A., Dillon P. and Delaby L. (2010) Producing milk from grazing to reconcile economic and environmental performances. *Grassland Science in Europe* 15, 865–879. Pinxterhuis J.B. (2011) *Report on appreciation of the current and future functions of grasslands in Europe and identification of implementation gaps between today and future multi-functionalities, as seen by international stakeholders*. Report Multisward. Wageningen UR Livestock Research, Lelystad, the Netherlands. Woodhill A.J., Van Vugt S.M., Van Geene J. and Verhoosel K.S. (2009) *The MSP guide: stakeholder collaboration for social innovation: designing and facilitating multi-stakeholder processes*. Wageningen International, Wageningen, the Netherlands. See also http://portals.wi.wur.nl/msp.

#### On the economics of bio-energy recovery from semi-natural grasslands

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#### Abstract

For the preservation of endangered species-rich grassland habitats, several extensive management options can be envisaged. However, structural changes in agriculture have led to a decline of customary animal keeping in many places. One potential land-use option could therefore be the use of semi-natural grassland material for renewable energy generation by the newly developed IFBB procedure (*Integrated Generation of Solid Fuel and Biogas from Biomass*) for renewable energy generation. This paper presents and models investment calculations for the preservation of semi-natural grassland; providing additional income and preserving agricultural enterprises in disadvantaged areas. This paper presents and models the investment for a commercially operated IFBB bio-energy system, producing electricity and grass pellets for heating purposes based on substrate from semi-natural grassland habitats. Several scenarios are illustrated. General frameworks and influencing parameters on economic performance of this alternative are also identified by sensitivity analysis.

Keywords: IFBB bio-energy system, investment calculation, economic key figures

#### Introduction

In some European regions a decline in the area of extensive grassland by up to 25% is anticipated in the medium-term future (Rösch et al., 2009), threatening the biodiversity and even the existence of many valuable semi-natural grassland habitats. A newly developed procedure for generating bio-energy, the Integrated Generation of Solid Fuel and Biogas from Biomass (IFBB), could help to preserve those valuable habitats by using them within an extensive management regime. The IFBB procedure can – as a unique feature compared to other bio-energy systems – use plant material with high fibre contents and low digestibility. Besides incorporating a screw press to separate solid from liquid matter, the IFBB bio-energy system contains a biogas fermenter and a `Combined Heat and Power' unit (CHP) to provide electricity from biogas and a drying and pelletizing unit to produce marketable grass pellets for combustion purposes (for a detailed description of the IFBB procedure see Wachendorf et al., 2009). Due to the novelty of this technique, no investment calculations to evaluate the economic prospects of this procedure have been carried out so far. This paper, for the first time, presents results of the economics of an up-scaled IFBB bio-energy system to give fundamental decision support to farmers, politicians and potential investors. Furthermore the aim of this paper is to identify and weight the importance of economic drivers by sensitivity analysis, influencing the profitability of the IFBB procedure.

#### Materials and methods

An economic simulation model of the IFBB system economics was constructed. Allocated average annual costs and revenues (total annuity) of an example IFBB unit were calculated by applying dynamic investment calculations according to the guidelines for *economy calculation systems for capital goods and plants* (VDI 6025, 2002). Key indicators of profitability were calculated according to Wöhe (2005): Return On Investment (ROI) as the Internal

Rate of Return (IRR), based on the gross Free Cash Flow (FCF); and Return On Equity (ROE) as the Internal Rate of Return to equity, based on the investor's dividend payouts from the net FCF. The calculations cover investment costs for feedstock storage, plant feeding, watering (conditioning) and dehydration of substrate, biogas production, power and heat generation, digestate storage, buildings and infrastructure, as well as costs for plant installation and planning and authorization. The system life-span was assumed to be 20 years, and the installed CHP has an electrical performance of 50 kW<sub>al</sub>. The main system parameters were determined according to multiple verified sources (expert interviews; relevant publications) (Table 1).

Table 1. Assumptions for the main variable IFBB b	oio-energy system parameters
Rate of pellet price increase per year: 4%	Proportion of equity of overall h

Rate of pellet price increase per year: 4%	Proportion of equity of overall budget: 40%
Selling price for solid fuel ex factory: 171 € Mg <sup>-1</sup>	Interest rate external finance: 6%
Two full-time employees (10 h d <sup>-1</sup> )	Substrate costs: 22 € ha <sup>-1</sup> (7 € t DM <sup>-1</sup> )
Labour wage (employer, gross): 15 € h <sup>-1</sup>	Annual grassland subsidies: 335 € ha a <sup>-1</sup>
Grassland yields: 3.8 Mg DM ha <sup>-1</sup>	Power reimbursement: EEG (2012), Germany

Grassland subsidies, including 85 € ha<sup>-1</sup> of direct payments and 250 € ha<sup>-1</sup> from agri-environmental schemes, are received by the grassland manager. Fixed plant parameters could be linked to three different models: grassland management is (i) executed by farmers (model 1), (ii) executed by the IFBB plant operator (investor or farmers' co-op), who rents farmland and outsources the grassland management to contractors (model 2), (iii) executed by the plant operator and 50% of substrate input is substituted by communal green cut material (model 3).

#### **Results and discussion**

Investment costs for the example IFBB system were calculated to be nearly 1.7 million €, with the main investments (>100.000 €/investment) for silage storage, extruder press, fixed bed fermenter and pelletizing unit. Considering the power generation of a 50 kW<sub>a</sub> CHP and methane yields of 406 normal litres (1) kg<sup>-1</sup> organic matter (oM) of the press fluid, semi-natural grassland requirements are at 510 hectares per year. Including both waste heat from the CHP and the thermal energy stored in the grass pellets (4.98 kWh kg<sup>-1</sup>) the system has a total thermal performance of 0.8 MW, with the main part (91.15%) coming from pellets, indicating the importance of pellet production in this procedure. Pellet yields account for 1502 Mg year-1, of which 15.22%, or 229 Mg, are burned in a combustion unit of the IFBB unit, mainly to meet the thermal energy demands for the drying process of the grassland press cake before pelleting. 80% of the produced electricity is needed as internal process energy consumption of the plant. The main annual expenses come from capital costs (44%), followed by costs for processing energy (27%), management (19%) and maintenance (7%) and substrates (3%). Substrate costs are low because of the very extensive grassland management and expense compensation by direct and agri-environmental payments. 82% of the annual income comes from grass pellets sales, and 18% from power sales (according to EEG 2012). Including the fertilizing values of digestate and ashes would further increase the annual income. Although displaying a lower profitability, model 1 is still profitable, since the ROI is above the external interest rate of capital and the annuity is positive (Figure 1). Model 2 has an increased profitability, since the grassland is managed by the plant operator, and therefore governmental grassland payments can also be credited to the silage production costs that will reduce them significantly. The further increase of profitability in model 3 can be explained by the additional use of communal green-cut



Figure 1. Economic key figures of the IFBB plant economics of three different models

(CGC) material. Although producing lower methane yields, pellet production is higher due to higher dry matter contents of CGC.

Based on the plant simulation model, economic drivers were identified (Figure 2).



Figure 2. Identified economic drivers and modification (%) of the ROE by economic driver variation of -10%

Economic drivers with the highest impact on changes in profitability, as identified by sensitivity analysis with a change of more than 20% of the ROE by an input variance of -10%, are pellet sales prices, grassland subsidies (affecting substrate costs) and investment costs.

#### Conclusions

The described IFBB bio-energy system would perform well, from an economic point of view, if there is a favourable mix of external circumstances and input parameters. The operation of an IFBB plant is especially suitable for regions with a high aggregation of readily available material. Considering freight charges, a strong regional or supra-regional demand for solid fuels is needed. An IFBB plant is able to create additional income for several stake-holder groups along the production chain, especially in agriculturally disadvantaged areas.

#### References

EEG (2012) Erneuerbare-Energien-Gesetz, Bundesgesetzblatt Teil I, 42, 1634 pp.

Rösch C., Skarka J., Raab K. and Stelzer V. (2009) Energy production from grassland – Assessing the sustainability of different process chains under German conditions. *Biomass and Bioenergy* 33, 689–700. VDI 6025 (2002) Economy calculation systems for capital goods and plants. Verein Deutscher Ingenieure. Wöhe G. (2005) *Einführung in die allgemeine Betriebswirtschaftslehre*, München, Franz Vahlen, 1220 pp. Wachendorf M., Richter F., Fricke T., Grass R. and Neff R. (2009) Utilization of semi-natural grassland through integrated generation of solid fuel and biogas from biomass. *Grass and Forage Science* 64, 132–143.

#### Sustainability of a regional feed centre

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#### Abstract

A feed centre buys crops from dairy farmers and arable farmers, processes them into total rations, and sells them to a number of dairy farmers to be delivered at the feeding fence. Feed centres are common practice in countries like Israel, but not in the Netherlands. This study aims to assess the sustainability of a regional feed centre on the basis of economics, traffic movements, energy consumption and landscape. Thanks to a feed centre, the cost price on dairy farms may decrease by  $\notin 1.80$  to  $\notin 3.30$  per 100 kg milk in the long term, depending on a regime of grazing or non-grazing and the quantity of land available. Even though the number of traffic movements increases when using a feed centre, the total energy consumption decreases. The farm surroundings improve as on-farm feed storage facilities will disappear and a greater diversity of crops is grown. Another advantage is that grassland management may improve because reliable data about the quality and quantity of grass will become available.

Keywords: economy, energy consumption, feed centre, landscape, sustainability, traffic

#### Introduction

The use of feed centres is common practice in Israel (Sprecher, 2007) and they are being introduced in countries like Denmark (Jensen, 2009), Korea, and Spain. A feed centre buys crops from dairy farmers and arable farmers, processes them with simple feedstuffs and minerals into total rations and sells them to a number of dairy farmers, to be delivered at the feeding fence. In addition to the supply of rations, the operations of the feed centre can also include nutritional and crop management support. Three entrepreneurs in the Netherlands started a first regional feed centre with six farms in 2010, and 28 farms (3000 cows) in 2011. They were inspired by the examples in Israel and Denmark. This study aims to assess the sustainability of a regional feed centre in the Netherlands on the basis of economics, traffic movements, energy consumption and landscape.

A feed centre offers many advantages (Galama and Beldman, 2010; Galama, 2011). The dairy farmer can contract part of his work. For several farms, on-farm feed storage is no longer necessary, as feed is stored centrally. A poor farm lay-out (land far away from the farm) is also less problematical as crops are brought to a central place. Separating the production and storage of feedstuffs from milk production leads to optimisation of the cultivation and feed supply regionally instead of on a farm level. The dairy farmer can grow the grass, maize, protein crops (such as lucerne or lupin) or concentrate feed replacers (e.g. cereals) at the best place. He can specialise in milk production. He will also be able to contract part of the crop cultivation to arable farmers. The feed centre then acts as a link between feed producers and dairy farms. This makes dairy farming land-related at a regional level. A feed centre can have a major impact on the income of feed producers and dairy farmers. A side-effect is that there will be more traffic movements.

#### Materials and methods

The sustainability of a regional feed centre has been assessed on the basis of economics, traffic movements, energy consumption and landscape. The cost-price calculations have

been done for five farm types, differing in number of cows, area and grazing system. The calculations about traffic and energy are made at a regional level for 15 farms (three of each type) with a total of 3300 dairy cows. The feed supply for the feed centre comes from these dairy farms (grass and maize) and arable farmers (concentrate replacers). Only a small part of the concentrates comes from the feed factory.

#### **Results and discussion**

The services of a feed centre and the additional costs of transport of grass and maize to the feed centre amount to  $\notin$ 25 per ton DM on the basis of 3300 dairy cows, a distance to the farms of 15 km and the processing of concentrate replacers from the region. The cost will be higher if fewer cows participate and if the distance to the farms is greater. These extra costs must be compensated for. Table 1 indicates the change in cost price for the five types of dairy farms.

Table 1. Long term change in cost price due to introduction of a feed centre for five types of dairy farms (€ per 100 kg milk), Sumf = summerfeeding

	Landless	Intensive	Intensive	Extensive	Extensive
	Sumf	Sumf	Grazing	Sumf	Grazing
Farm type	1	2	3	4	5
Number of cows	500	150	150	150	150
Area in ha	0	75	75	150	150
Grazing system	n.a.	none	limited	none	ad lib
Change in total costs	-2.9	3.9	2.3	5.6	2.7
Feed + transport	1.3	9.0	5.5	10.1	5.6
Cattle, crops, energy and other	-0.3	-0.4	-0.2	0.7	0.2
Labour	-0.6	-0.9	-0.5	-0.9	-0.5
Contract work	-1.1	-1.3	-0.8	-2.1	-1.1
Feed storage	-1.5	-1.6	-1.1	-1.4	-0.8
Mechanisation of feeding	-0.3	-0.8	-0.7	-0.8	-0.7
Manure removal	-0.3	-0.2			
Additional sale of feed	0.0	7.2	4.5	7.7	4.5
Change in cost price	-2.9	-3.3	-2.1	-2.1	-1.8

The lower cost price for the landless farm (type 1) is primarily a result of the lower costs of feed storage and of contract work to carry the feed into the trench silos. Because the feed centre brings the rations up to the feeding fence, the costs of labour and mechanisation for the feeding process will be less. Farm types 2 to 5 demonstrate major changes in the costs of feed and in the sale of feed. The cost of feed refers to both forage and concentrate feed. In situations with a feed centre, concentrates are replaced by wheat and Maize Crop Silage (MCS). In the end, the advantage varies between €3.30 (intensive summer feeding) and € 1.80 (extensive and frequent grazing) per 100 kg milk. This is 5 to 10% of the total cost price. The advantage under grazing is smaller as there are fewer savings on feed storage. The short-term advantage for farms will depend on the individual farm situation. When farmers have invested in feed storage, the feed centre will be less profitable. Furthermore, there are major differences in feed efficiency at the farm. If a sophisticated ration from the feed centre raises the feed efficiency or the protein content in milk, this will result in a direct economic profit (cash flow). Furthermore, the feed centre may buy ingredients or by-products at lower prices than individual dairy farmers. As all yields of crops (mostly grassland) and feeding efficiencies are measured, the suppliers of feed and buyers of total mixed ration are given insight into their farm management. Knowledge about grassland management will
increase because reliable data about the quality and quantity of grass will come available. These advantages have not been considered in the calculations.

The number of traffic movements will be affected by the feed centre. The basic situation assumes a general scaling-up with many tractor movements from field plots to the farmyard. With a feed centre, transport will be performed by trucks. On a daily basis, the trucks collect the forage from the dairy farms and other locations in the region and deliver the rations to the dairy farms. The number of tractor movements in the basic situation is over 8300, whereas the number of truck movements in the situation with a feed centre is over 14000. The energy consumption consists of energy consumption on dairy farms, at the feed centre and in transport. The additional energy consumption due to the higher transport requirements has been weighed against energy saving on the farm and by growing more feedstuffs in the region. In the situation with the feed centre, the total energy consumption decreases by 9%. This saving is achieved partly because the import of raw materials from abroad for concentrate production is replaced by regional cultivation of concentrate replacers such as wheat or MCS. Centralisation of feed storage and mechanisation also saves energy. The disadvantage of additional transport of crops to the feed centre and of rations to the dairy farms is small.

As a consequence of the central feed storage, the on-farm feed storage facilities disappear from the landscape. More space becomes available on the farm. With the central feed storage, the cropping plan of arable farms will become more varied as a greater diversity of crops is grown. Whether there are good prospects for a feed centre depends on several conditions. The location should avoid causing danger on public roads. The scale should be large enough, implying that sufficient farms should be willing to participate. Proper agreements should be made with the participants about prices, cropping plans, rations and coverage of risks due to poor quality or calamities (e.g. disease outbreak). A decision also has to be made about the financing structure. The feed centre could be owned and run by an independent enterprise or as a farmers' cooperative (dairy farmers and arable farmers).

## Conclusions

Analyses of prospects for a regional feed centre show that introduction of a feed centre will lead to increased sustainability due to a lower cost price of the dairy farms and arable farms, less total energy use and a better landscape. The grassland management may improve because reliable data about the quality and quantity of grass will become available.

#### References

Galama P. (2011) *Prospects for a regional feed centre*. Book 15. Wageningen UR Livestock Research, the Netherlands, 39 pp.

Galama P. and Beldman A. (2010) *Duurzaamheid regionaal grondgebonden grootschalige melkveehouderij*. Report 424, Wageningen UR Livestock Research and LEI, the Netherlands, 53 pp.

Information on the pilot in Friesland: www.voercentrum.nl

Jensen E.B. (2009) The arable farmer in feed centres providing TMR rations (Denmark). Dansk, Landbrugsrådgivning Landscentret, Okonomi og Jura.

Sprecher Y. (2007) Agro parks for dairy farmers. Centralized Multi-Farm projects. Sprecher architects, Tel Aviv, Israel.

# Analysis of economic aspects of the implementation of the agri-environmental programme on permanent grassland in Poland

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# Abstract

The objective of this study was the analysis of economic aspects of the implementation of selected measures of the agri-environmental programme in Poland dedicated to permanent grassland. The performed analysis revealed considerable disproportions in the utilization of resources between individual measures. In 2010, extensive permanent grasslands, as Measure 3, attained the target value at over 108%, whereas Measures 4 and 5 (Protection of endangered bird species and natural sites on and outside the NATURA 2000 areas, respectively) despite significantly higher subsidies per hectare, were completed at only 7% of the value expected. This can be attributed to more stringent requirements regarding the application and implementation of Measures 4 and 5.

Keywords: economy, permanent grassland, agri-environmental programme

# Introduction

Permanent grasslands constitute a significant element of the natural landscape in Poland and play an important role both in agriculture as well as in the environment. On average, they make up 20% of the total structure of utilised agricultural area (UAA) in our country (GUS, 2011). Moreover, permanent grasslands constitute almost 50% of farmland covered by the European Ecological Network Natura 2000. In order to disseminate the model of sustainable farmland management, the European Union implements in member countries various forms of financial support, among others, agri-environmental programme (AEP). The first phase of the project was realised in Poland in years 2004–2006. In the study presented here, the performed analysis deals with the process of distribution of the EU financial aid within the framework of the current AEP for the years 2007–2013 on areas occupied by permanent grasslands.

# Materials and methods

The following three measures dedicated to permanent grasslands were taken into consideration when analysing economic aspects of the implementation of the AEP constituting part (Action 214) of the Rural Area Development Programme (RADP): (i) Measure 3. Extensive permanent grasslands; (ii) Measure 4. Protection of endangered bird species and natural sites outside Natura 2000 areas, and (iii) Measure 5. Protection of endangered bird species and natural sites within Natura 2000 areas. The principal source of primary data was derived from information obtained from the Ministry of Agriculture and Rural Development gathered in the process of application, verification and requesting for financial support within the RADP (Report, 2011). In addition, data from the Central Statistical Office (GUS, 2010) as well as available literature on the subject were used. The performed analyses comprised years 2008 to 2010.

# **Results and discussion**

The first intake of applications and distribution of payments within the framework of the AEP 2007–2013 took place in 2008. Measures 3, 4 and 5 adopted a more detailed form in comparison with

the edition of the programme realised in years 2004–2006. Moreover, the level of payments depending on the realised variant of the measure in a given region also changed. The level of subsidies allocated to meadows and pastures in the action comprising years 2004–2006 ranged from 400 to 1030 PLN ha<sup>-1</sup>, whereas in the case of the 2007–2013 AEP, the payments earmarked for the same measures ranged from 500 to 1390 PLN ha<sup>-1</sup> (mean National Bank of Poland EUR/ PLN exchange rate on 15.11.2011 = 4.4064). Taking into consideration the average structure of private farms (Figure 1) in which meadows and pastures make up nearly 20% of the land area, it can be said that subsidies paid to these areas exert a significant influence on the economic situation of this group of farms, as they occupy 96.2% of all UAA in Poland.

Advantages gained from the realisation of a given measures of the AEP on permanent grasslands may exert an exceptional influence in the case of small agricultural farms. In 2011, the average area of an agricultural farm in Poland amounted to 10.36 ha. The average income from work per one conversion hectare amounted to 2278 PLN. When analysing the course of the execution of the AEP for 2007–2013, it is necessary to take into account the sum of realised payments as well as the number of farms that submitted applications for a subsidy in a given year (Table 1).



Figure 1. Land use in agricultural farms of private sector (GUS, 2011)

Table 1. Amounts of realised payments (PLN) and beneficiaries numbers of selected measures of agri-environmental programme in 2008–2010 in Poland

	Selected measures of agri-environmental programme						
-	Measur	e 3	Measur	re 4	Measu	re 5	
Year	Amount	Beneficiar-	Amount	Beneficiar-	Amount	Danafiaiariaa	
	of realised	ies	of realised	ies	of realised	Deficition	
	payments	number	payments	number	payments	number	
2008	22 188 054.96	n/a	0.00	0	0.00	0	
2009	55 470 277.20	12 435	1 286 048.43	77	3 308 006.37	118	
2010	115 618 819.68	23 079	10 347 432.11	453	23 732 964.22	626	

During the first year of operation of the programme, only payments within the framework of Measure 3 were realised (Liro, 2010). This was connected with the requirement to submit additional ecological documentation by farmers applying for subsidies from Measures 4 and 5. This delay can be eliminated by the inclusion of a given grassland area into Measure 3 and, after the submission of the required certificates and documentation, its re-evaluation and assignment to Measure 4 or 5. However, the discussed three measures cannot be applied jointly. The first applications for subsidies of naturally valuable areas were approved in the year 2009, although Measure 3 continued to dominate and its value doubled in relation to the previous year. The same dynamics was recorded in year 2010 (Figure 2).

Year 2010 reflects most faithfully the effects of the realisation of the AEP with respect to the scale of granted subsidies and numbers of beneficiaries. Measure 3 – Extensive permanent grasslands – continued to be the dominant measure within which payments were



Figure 2. Dynamics of changes in realised payments of selected measures of agri-environmental programme in 2008–2010 (Mio. PLN)

paid out. Farms committed to the participation in this programme, on average, were paid out a sum of 5009.7 PLN. In addition, this measure had the highest share in the total sum of the entire AEP, reaching the level of 20%.

The year 2010 also showed a growing interest in Measures 4 and 5. The number of beneficiaries increased several-fold. Due to higher unit payments per hectare in comparison with Measure 3, measures aiming to protect birds' hatching sites and areas generating higher income to farms. Variants 4.1 and 5.1 "Protection of birds' hatching sites" characterised by the highest unit quota among the discussed action plans, constituted 80–90% of payments in both measures. In 2010, the mean quota paid out to farms realising this programme within the framework of Measure 4 or Measure 5 amounted to 22842 PLN, and 37912 PLN, respectively. Disproportions between these measures are also apparent in numbers of beneficiaries as well as the area of permanent grasslands included in the programme. Measure 3 was assumed to cover an area of 190 000 ha of meadows and pastures. In 2010, the target value of the measure was realised at 108%. Analysing the assumptions of Measure 4 (220 000 ha) and Measure 5 (370 000 ha), the targets were realised at only 7% of value expected.

## Conclusions

The implementation of the 2007–2013 AEP on areas of permanent grasslands progresses successfully as confirmed by the increasing trends in the dynamics of new beneficiaries as well as of utilised resources. What raises concern is the considerable disproportions between measures, in particular Measure 3 vs. Measures 4 and 5. It can be concluded that beneficiaries prefer to accept lower but certain payments for extensive management on meadows and pastures, and they deliberately avoid the possibilities for considerably higher subsidies for environmental measures, which require additional procedures at the level of submitting applications and implementation.

## References

GUS (2010) *Statistical Yearbook of Agriculture 2010*. Main Statistical Office, Warsaw, pp. 389. Liro A. (2010) Conditions of natural environment in rural areas. In: Wilkin J. and Nurzyńska I. (eds) *Rural Poland 2010*, Rural Development Report, Foundation for the Development of Polish Agriculture, Warsaw, pp. 95–120.

Report (2011) Report of realization of activities within Rural Area Development Programme 2007–2013. Ministry of Agriculture and Rural Development, Warsaw, No. 4/2010, pp. 71–79.

# An economic comparison of dairy production on N fertilized and white clover grassland

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# Abstract

This study compared the profitability of dairy production on N-fertilized grass (FN) and grass-white clover (WC) grassland and assessed sensitivity to fertilizer N and milk prices. Data were sourced from three system-scale studies conducted in Ireland between 2001 and 2009. Ten FN stocked between 2.0 and 2.5 Livestock units (LU) ha<sup>-1</sup> with fertilizer N input between 173 and 353 kg ha<sup>-1</sup> were compared with eight WC stocked between 1.75 and 2.2 LU ha<sup>-1</sup> with fertilizer N input between 79 and 105 kg ha<sup>-1</sup>. Sensitivity was confined to nine combinations of high, intermediate and low fertilizer N and milk prices. Stocking density, milk and total sales from WC were approximately 0.90 of FN. In scenarios with high fertilizer-N price combined with intermediate or low milk prices, WC was more (*P* < 0.05) profitable than FN. Between 1990 and 2005 FN was more profitable than WC. However, with the increase in fertilizer N prices relative to milk price, the difference in profitability between FN and WC was less clear cut between 2006 and 2010. Projecting into the future and assuming recent trends in fertilizer N and milk prices, WC will become an increasingly more profitable alternative to FN for grazed grassland-based dairy production.

Keywords: white clover, fertilizer N, grassland, dairy production, net margin

# Introduction

Over the last decade, the farm-gate cost of fertilizer N in Ireland has been increasing at an annual rate of around 9%. In contrast, milk price, while variable, has been relatively static (CSO, 2010). This is negatively impacting on profitability of pasture-based dairy production, which is highly reliant on fertilizer N. Hence there is a need to evaluate the potential of white clover to replace fertilizer N and contribute to the profitability. This study compared the profitability of systems of dairy production based on N-fertilized grass (FN) and grass-white clover (WC; annual average of ca. 0.23 white clover in herbage DM) grassland. Data from three system-scale studies were combined with recent farm-gate prices to determine relative profitability in the context of recent and changing fertilizer N and milk prices.

# Materials and methods

Production data were derived from three previous studies (Humphreys *et al.*, 2008; 2009; Keogh *et al.*, 2010) conducted at Solohead Research Farm (52°51'N, 08°21'W) between 2001 and 2009. There were one WC and three FN in 2001 replicated in 2002, one WC and one FN in 2003 replicated in 2004, 2005 and 2006, and one WC in 2008 replicated in 2009. Therefore, for the present study, there were ten FN and eight WC involving a range of stocking densities of spring-calving Holstein-Friesian dairy cows, inputs of fertilizer N and concentrate feed (Table 1). The production data were compared on the basis of a farm area of 50 ha, with dairy replacements reared and grass-silage produced on the farm to meet the winter forage requirements. Replacement rate

was on average 23 per cent. Surplus calves were sold once they were approximately 3 weeks old and culled cows were sold at the end of lactation, in December each year. Approximately 0.90 of the diet was home-grown forage, approximately 0.65 grazed pasture, 0.25 grass-silage and 0.10 concentrate, in line with that recorded in the above experiments. For the economic interpretation of the physical data, data from the Central Statistics Office of Ireland (CSO, 2010) and Anon. (2008) were used. Fixed costs were taken from Ramsbottom and Clark (2010). The net margin per hectare was calculated using a spreadsheet model developed in Excel. Sensitivity analysis (Kleijnen, 1997) was carried out to investigate the net margin response to changing fertilizer N and milk prices. There were three fertilizer N prices ( $\& kg^{-1}$  N): low (0.68 for urea and 0.86 for calcium ammonium nitrate [CAN]), intermediate (0.83 for urea and 0.98 for CAN) and high (0.95 for urea and 1.35 for CAN) (CSO, 2010). Similarly, for milk there were three prices (& litre<sup>-1</sup>): low (0.23), intermediate (0.29) and high (0.34). Nine scenarios were investigated including all combinations of fertilizer N and milk prices described above. Data were subjected to analyses of variance to compare differences in production factors, sales, costs, gross and net margins per ha between systems with data from systems in individual years as replicates.

Table 1. Characteristics of the systems of dairy production based on N-fertilized grass (FN) and grass-white clover (WC) grassland at Solohead Research Farm between 2001 and 2009 (Humphreys *et al.*, 2008; 2009; Keogh *et al.*, 2010). Data are means of two and four years

	WC		FN		WC	FN	WC
Year		2001	to 2002		2003 t	o 2006	2008 to 2009
Stocking density (cows ha <sup>-1</sup> )	1.75	2.10	2.50	2.50	2.15	2.15	2.12
Fertilizer N (kg ha-1)	80	180	248	353	90	225	100
Concentrate (kg cow <sup>-1</sup> )	535	535	535	535	525	525	575
Milk output (kg cow <sup>-1</sup> )	6550	6275	6242	6375	6521	6526	6273

#### **Results and discussion**

Stocking density, milk, cull cow, and calf sales per ha from WC were approximately 0.90 (P < 0.05) of FN (Table 2). Total variable costs of WC were approximately 0.82 (P < 0.05) of FN due to lower fertilizer-N costs associated with the replacement of fertilizer N by biologically fixed N and to the smaller scale of production on WC leading, for example, to lower contractor charges. Most (0.58) of the difference in variable costs was due to differences in the fertilizer N input; the remainder was mostly due to differences in scale.

There was no difference in gross margin between the systems. Fixed costs were higher (P < 0.05) on FN, which was attributable to activities associated with higher stocking densities and higher milk output such as electricity use, labour and repayments on capital investments, which, in general, tend to increase per ha with increasing scale. There was no difference in net margin between the systems for the scenario with intermediate milk and fertilizer N prices.

In the sensitivity analysis the general trend was that net margins of FN and WC were very sensitive to changing milk price and to a much lesser extent to changing fertilizer N price (Table 2). In scenarios with high fertilizer-N price combined with intermediate or low milk prices, or intermediate fertilizer-N price combined with low milk price, WC was more (P < 0.05) profitable than FN. In contrast, where low fertilizer-N price was combined with high milk price FN was more (P < 0.05) profitable than WC. Taking into account milk and fertilizer-N prices at the time, FN was more profitable than WC between 1990 and 2005, which is in general agreement with many previous studies (Doyle *et al.*, 1984; Ryan, 1988; Penno *et al.*, 1996; Schils *et al.*, 2000; Leach *et al.*, 2000). However, with the steady increase in fertilizer-N prices relative to milk price, the difference between FN and WC was less clear cut between 2006 and 2010, with no difference between systems in 2006 and 2008, FN more profitable in 2007 and 2010 and WC more profitable in 2009.

System		FN	WC	P Value
Stocking density (LU ha <sup>-1</sup> )		2.28	2.04	< 0.05
Fertilizer N (kg ha-1)		246	90	< 0.001
Milk sales (€ ha <sup>-1</sup> )		3168	2875	< 0.05
Total sales (€ ha <sup>-1</sup> )		3530	3205	< 0.05
Fertilizer N (€ ha <sup>-1</sup> )		223	75	< 0.001
Total variable costs (€ ha <sup>-1</sup> )		1400	1146	< 0.01
Gross margin (€ ha <sup>-1</sup> )		2131	2058	ns
Fixed costs (€ ha <sup>-1</sup> )		860	781	< 0.05
Net margin (€ ha <sup>-1</sup> )		1271	1278	ns
Fertilizer N price	Milk price	Net Marg	in (€ ha <sup>-1</sup> )	
Н	Н	1761	1751	ns
Н	М	1202	1252	< 0.05
Н	L	597	703	< 0.01
М	Н	1812	1779	ns
М	М	1271	1278	ns
М	L	657	714	< 0.05
L	Н	1845	1775	< 0.05
L	М	1294	1275	ns
L	L	690	726	ns

Table 2. The economic performance of systems of dairy production based on N-fertilized grass (FN) and grass-white clover (WC) grassland at intermediate fertilizer N and milk prices, and the impact of high (H), intermediate (M) and low (L) fertilizer N and milk prices on net margin per ha

#### Conclusions

Projecting into the future, assuming similar trends in fertilizer N and milk prices to that in the last decade, this analysis indicates that WC is likely to become an increasingly more profitable alternative to FN for grazed grassland-based dairy production.

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#### References

Anonymous (2008) Management data for farm planning. Teagasc, Oak Park, Carlow, 296 pp.

Central Statistics Office (2010) Agricultural input and output absolute prices. Central Statistics Office, Cork. Doyle C.J., Morrison J., Brockman J.S., Camm B.M. and Reeks J. (1984) Economic aspects of using legumes. In: Thompson D.J. (ed.). *Forage Legumes*. Hurley, UK, British Grassland Society, pp. 152–165. Humphreys J., Casey I.A. and Laidlaw A.S. (2009) Comparison of milk production from clover based and fertilizer N based grassland on a clay-loam soil under moist temperate climatic conditions. *Irish Journal and Agricultural and Food Research* 48, 189–207.

Humphreys J., O'Connell K. and Casey I.A. (2008) Nitrogen flows and balances in four grassland based systems of dairy production on a clay-loam soil in a moist maritime environment. *Grass and Forage Science* 63, 467–480. Keogh B., Humphreys J., Phelan P., Necpalova M., Casey I.A. and Fitzgerald E. (2010) The effect of organic management strategies on dairy production in clover based grassland. *Grassland Science in Europe* 15, 958–960. Kleijnen J.P.C. (1991) Sensitivity analysis and related analyses: a review of some statistical techniques. *Journal of Statistical Computation and Simulation* 57, 111–142.

Leach K.A., Bax J.A., Roberts D.J. and Thomas C. (2000) The establishment and performance of a dairy system based on perennial ryegrass-white clover swards compared with a system based on nitrogen fertilized grass. *Biological Agriculture and Horticulture* 17, 207–227.

Penno J.W., MacDonald K.A. and Bryant A.M. (1996) The economics of the No. 2 dairy systems. *Proceedings of 48<sup>th</sup> Ruakura Farmers Conference*, Hamilton, New Zealand, 11–19.

Ramsbottom G. and Clark P. (2010) Profit monitor update for spring calving herds. Teagasc, Oak Park, Carlow. Ryan M. (1988) Development of a legume based dairy system. In: Plancquaert P. and Haggar R. (eds). *Legumes in Farming Systems*, EEC, Brussels, 159–167.

Schils R.L.M., Boxem T.J., Jagtenberg C.J. and Verboon M.C. (2000) The performance of a white clover based dairy system in comparison with a grass/fertilizer N system. II. Animal production, economics and environment. *Netherlands Journal of Agricultural Science* 48, 305–318.

# International transfer of forage information and concepts

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## Abstract

In 1991, the first edition of the book 'Southern Forages' was published, with second, third, and fourth editions published in 1996, 2002, and 2007, respectively. Each successive edition since the first one has been updated and expanded from the previous one. It has filled a major void because a comprehensive, practically-oriented and well-illustrated forage book was not readily available. The book has been widely accepted and used by livestock producers, seed, chemical, fertilizer, and equipment dealers; agricultural extension and natural resources personnel; vocational teachers; and consultants. It has been extensively used in grazing schools, in various types of short courses, and as a text in over 60 colleges and universities. Though the name implies a Southeastern United States orientation, it has been distributed throughout the USA and more than 30 other countries, plus it has been translated into Polish, Chinese, Czech and Spanish with other translations in progress. Other items based on the book have been developed as well including: a publication titled 'Forage Crop Pocket Guide, Ten Keys to a Profitable Forage Program and posters on Forage Grasses and Forage Legumes.' Several USA Seminars have been developed around southern forage principles and a DVD on Keys to Forage Profitability has been produced.

Keywords: forage, legumes, grasses, books

## Introduction

Rather than providing results from experiments, this paper provides a discussion of an approach that has been successfully used to disseminate forage information. Knowledge of forage production concepts and a way to locate practical forage information are needed by producers of grazing animals. Yet many forage publications are either too technical for producers to understand or have a narrow focus in terms of subject matter and/or geographical application. In 1985, authors of this paper first discussed the need for a comprehensive, practically-oriented forage reference book because no such publication was then available. This led to development of an outline and to initiation of work on writing such a publication.

From the beginning, it was felt that the book should provide information on adapted forage species commonly grown in the Southeastern USA, complete with colour photographs of each one. It was also felt that there should be chapters on basic production topics such as soil testing and fertility, seed, establishment, and legume inoculation as well as on forage physiology, forage ecology, and forage quality. It was further decided there should be chapters on nutrient requirements of livestock, common forage livestock disorders, grazing management, as well as chapters dealing with specific livestock species including beef cows and

calves, beef stockers, dairy cattle, horses, and even wildlife. Furthermore, the decision was made to include a comprehensive appendix to facilitate providing information of the type that most people do not normally remember.

Once a first draft of the publication had been put together, the authors contacted personnel associated with the Potash & Phosphate Institute (now the International Plant Nutrition Institute or IPNI) and asked if they would be willing to publish the book. They agreed to do so, which was fortunate because they had extremely talented employees to assist with editing, layout, and other aspects of the publication.

# Discussion

In 1991, the first edition of the book 'Southern Forages' was published, and second, third, and fourth editions of the book were published in 1996, 2002, and 2007, respectively. The response to the book has been extremely gratifying. It has been widely accepted and used by livestock producers, seed, chemical, fertilizer, and equipment dealers; agricultural extension and natural resources personnel; vocational teachers; and consultants. It has been extensively used in grazing schools, in various types of short courses, and as a text in over 60 colleges and universities. To date, over 50,000 have been printed. Though the name implies a Southeastern United States orientation, orders for it have come from all over the USA and from more than 30 other countries, plus it has been translated into Polish, Chinese, Czech and Spanish with other translations in progress.

Four other items based on the book have been developed as well. A publication titled 'Forage Crop Pocket Guide', that consists mainly of tables and figures from the book, was published by IPNI in 1999, and 70,000 have been printed. In addition, posters on Forage Grasses and Forage Legumes that contain colour pictures of various species discussed in greater detail in the 'Southern Forages' book, are particularly useful in educational settings and seed outlets. Concepts from the book (Keys to Forage Profitability) were summarized into a suitable-for-framing document and in 2011 a DVD was produced and released.

*Lectures/Seminars*: Southern Forages lectures/ seminars based on principles in the book have been presented in numerous states within the USA and in several other countries.

## Conclusions

The popularity and wide use of this book suggest that the forage/livestock principles covered in Southern Forages have no geographic boundaries. It also provides evidence that if there is truly a need for an educational item, meeting that need may have more dramatic results than initially expected. There is no doubt that this publication, which the authors originally had expected to be of benefit mainly to beef cattle producers in the Southeastern United States, has truly had a worldwide impact. For more information, see the IPNI website www.ipni.net/sf.

## References

Ball D.M., Hoveland C.S., Lacefield G.D. and Nowak Cz. (1996) *Nowoczesne Technologie Produkcji Pasz Objetosciowych (Polish translation of Southern Forages)*. Demar Publishers, Krakow, Poland.

Ball D.M., Hoveland C.S., Lacefield G.D. and Li X. (2001) Southern Forages (Chinese translation of Southern Forages), China Agricultural Press, Beijing.

Cermak B., Ball D.M., Hoveland C.S. and Lacefield G.D. (2004) *Cultivation and use of bulky animal feed and environmental protection*. Scientific technical publications issued under the Ministry of Agriculture project / IAFI, ISBN: 80-7040-745-X.

Cermak B., Ball D.M., Hoveland C.S. and Lacefield G.D. (2004) *Effect of feed on production quality and wholesomeness of milk and meat.* Scientific technical publications issued under the Ministry of Agriculture project / IAFI, ISBN: 80-7090-744-1.

Lacefield G.D., Ball D.M., Hoveland C.S. and Armstrong D.L. (2008) A Forage Book of International Impact. *Proceedings of the XXI International Grassland Congress*, Vol II, 878.

# Potential sewage sludge utilization to fertilize forage surfaces in Galicia

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# Abstract

Sewage sludge is an extensively used fertilizer in Galicia, where more than 70% of the sewage sludge produced is used in agriculture. Nevertheless, before using sewage sludge as a source of nutrients for soil, special attention has to be paid to its content of heavy metals, which could be quite high. For this reason, there are European and Spanish regulations that take account of the levels of heavy metals in soils, in sludge and the maximum amount of heavy metals that can be applied for a period of ten years. Based on previous evaluation at the Spanish scale, Zn is the regulated heavy metal with the highest levels in the sludge. In the present study, a survey of around 2600 soils was performed in order to evaluate the levels of heavy metals in soils and the implications of these levels on the potential use of sewage sludge. Sewage sludge could be used in more than 94% of the analysed soils. Ni was the most limiting heavy metal for sludge application, mostly associated with soils that have serpentine rock as the parent material.

Keywords: heavy metal, biosolid, grassland

## Introduction

The production of municipal sewage sludge in Europe has increased since the start of the 1990s due to the implementation of the 91/271/CEE Directive, which makes the progressive purification of municipal sewage compulsory in municipalities. The residue from sewage treatment should be recycled as fertilizer as it has a good proportion of N, P and organic matter. However, the higher proportion of heavy metals in these residues, compared with the soil, needs the development of adequate regulations to reduce potential negative impacts of these heavy metals on soils and crops, and therefore for animals and human health. The European Union sewage sludge working document (EU 2000-Brussels, 27 April 2000 -ENV.E.3/LM) provided some rules to control the use of sewage sludge based on the heavy metal contents in the sludge, in the soil, and on the maximum quantity of different heavy metals that could be accumulated over a 10-year period on a field. Zn, Cu, Hg, Cd, Cr, Pb and Ni are the current heavy metals to be evaluated to determine the suitability of a soil to receive sewage sludge as fertilizer. The implications of the levels of heavy metals of the sludge and of corresponding policy rules were previously evaluated in Spain by Mosquera-Losada et al. (2010). Main conclusions indicated that, taking into account the sludge working document, it will not be possible to use sludge as a fertilizer in the future, unless the concentrations of Cd, Pb, Zn, and Hg in the sludge are reduced. In this paper, we compared the levels of heavy metals of 2597 soils to be used for forage production and the limits described in the current sewage sludge Spanish regulations based on Directive 91/271/EEC.

# Materials and methods

The study was carried out in Galicia, a region of more than three million hectares in the north-western part of Spain. It is localized in the south-western part of the Atlantic

bio-geographic region of Europe. From 2007 to 2010, 2597 soil samples, which had previously never been fertilized with sewage sludge, were randomly sampled to define if they were suitable to receive sewage sludge. Soil depth sampled was 25 cm, as established by the Spanish Royal Decree 1310/90. Once taken, all soil samples were transported to the laboratory and air dried. Afterwards, soil samples were sieved through a 2 mm sieve and digested using a microwave (CEM) with nitric acid. Subsequently, concentrations of Ni, Cd, Zn, Cr, Cu and Pb were measured by using an atomic spectrophotometer, and Hg with a hydride generator (VARIAN VGA-76). Soil pH was also measured in a water suspension (1:2.5).

## **Results and discussion**

Soil pH ranged from extremely acid to basic (Table 1), but most of the soils had a pH below 7 (only 38 soil samples had a water pH above 7). Heavy metal availability is usually higher in acid soils (Smith, 1996). This underlines the importance of evaluating the concentration of heavy metals in Galician soils. All mean concentrations of heavy metals (Table 1) were below the limits given by the Spanish regulation (Royal Decree 1310/90), but most of the maximum values were above this level with the exception of Hg. Zn and Hg concentrations were within the usual range for soils described by Davies (1980) which were between 10 and 300 mg kg<sup>-1</sup> for Zn and between 0.01 and 2 mg kg<sup>-1</sup> for Hg, while Cr (1.4–1389 mg kg<sup>-1</sup>) and Pb (3–189 mg kg<sup>-1</sup>) were within those given by Kabata-Pendías and Pendías (1985). However, some concentrations were above the soil limits considered as usual for Ni (10–100 mg kg<sup>-1</sup>), Cd (0.07–1.1 mg kg<sup>-1</sup>) and Cu (10–80 mg kg<sup>-1</sup>) given by Carter (1993), Kabata-Pendías and Pendías (1985) and Davies (1980), respectively.

	Value	Min Mor	Maan	CD.	Spanish regulation
Property		Iviin-iviax	Mean	SD	Spanish regulation
pH–H <sub>2</sub> O		3.44-10.22	5.21	0.72	
Cd (mg kg <sup>-1</sup> )		0.01-2.50	0.05	0.15	1
Ni (mg kg <sup>-1</sup> )		0.001-169.50	12.20	14.28	30
Pb (mg kg <sup>-1</sup> )		0.01-118.50	10.21	12.13	50
Zn (mg kg <sup>-1</sup> )		0.01-306.70	45.33	30.46	150
Hg (mg kg <sup>-1</sup> )		0.01-0.80	0.054	0.05	1
Cr (mg kg <sup>-1</sup> )		0.01-236.94	10.03	16.54	100
Cu (mg kg <sup>-1</sup> )		0.01-212.00	16.38	17.98	50

Table 1. Mean, minimum (Min) and maximum (Max) values and their standard deviation (SD) of water pH and heavy metals of 2559 soils tested in this experiment

Table 2 shows the number of samples below and above the limits defined by the Spanish regulation and Directive 91/271/EEC. None of the basic soils had heavy metal levels above the current limits. The heavy metal most limiting a possible sewage sludge application, in 6% of the sampled acid soils, was nickel. This could be explained by the occurrence of serpentine as parent rock material (Reeves *et al.*, 1999). Copper followed by zinc and lead concentrations in some soils were also above the Spanish limits. However, more than 99% of soil samples fulfilled the current Spanish regulations, except for Ni content. The rainfall regime in the region explains the acidity of the soil. This could also lead to heavy metals leaching and so to the observed concentrations below the current regulations.

	Legal require	ments for 11	se of sew	age sludg	e in soils Samn	les below/above the					
	Legar require	segar requirements for use of sewage studge in sons. Samples below/above the									
				limits							
		R.D.	1310/199	0 (Direct	ive 91/271/EEC)						
	pH ≤	7; $n = 2559$	9		pH > 7; n	= 38					
	limit	below	above	limit	below	above					
Cd (mg kg <sup>-1</sup> )	1	2557	2	3	38	0					
Ni (mg kg <sup>-1</sup> )	30	2408	151	112	38	0					
Pb (mg kg <sup>-1</sup> )	50	2548	11	300	38	0					
Zn (mg kg <sup>-1</sup> )	150	2540	19	450	38	0					
Hg (mg kg <sup>-1</sup> )	1	2559	0	1.5	38	0					
Cr (mg kg <sup>-1</sup> )	100	2550	9	150	38	0					
Cu (mg kg <sup>-1</sup> )	50	2542	55	210	38	0					

Table 2. Number of samples for each heavy metal with values above Spanish and EEC current directives depending on pH

## Conclusions

More than 94% of the analysed Galician soils fulfilled the current Spanish and European limits allowing the application of sewage sludge to soils. Nickel was the heavy metal that was the most limiting for sewage sludge application, and was usually associated with the presence of soil which had serpentine as parent material.

#### References

Carter M.R. (1993) Soil sampling and methods of analysis. CRC Press, LLC. Boca Raton, Florida, USA. Davies E.D. (1980) Applied soil trace elements. Wiley and Sons, Inc.

Kabata-Pendías A. and Pendías H. (1985) *Trace elements in soil and plants*. CRC Press (ed.). Florida, USA, 315 pp.

Mosquera-Losada M.R., Muñoz-Ferreiro N. and Rigueiro-Rodríguez A. (2009) Agronomic characterization of different types of sewage sludge: policy implications. *Waste Management* 30, 492–503.

Reeves R.D., Baker A.J.M., Borhidi A. and Berazaíns R. (1999) Nickel hyperaccumulation in the serpentine Flora of Cuba. *Annals of Botany* 83, 29–38.

Smith S.R. (1996) Agricultural recycling of sewage sludge and the environment. CAB International, Wallingford.

# The impact of roughage quality and production strategies on the milk yield from roughage in German organic dairy farms

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# Abstract

The milk yield from roughages is regarded to have a significant impact on the economic success and the sustainability of dairying (Rougoor *et al.*, 1999). In organic dairy farms the role of milk yield from roughages is even more important compared to conventional farms, due to the restricted application of concentrates and for economic reasons. In order to analyse the effect of production strategies on the milk yield from roughages, 106 organic dairy farms throughout Germany were grouped on the basis of their forage system. The area of grassland, grass-clover ley, maize, and the amount of concentrates were the main determining factors. Cluster analyses detected six different forage cropping-strategy types (FCST). Milk yield from roughages differed significantly among these six types. Further analyses revealed a high variability of roughage qualities among these types, due to different site conditions and varying forage management. The results are presented and discussed with special regards to the function of forage production in organic dairy farming systems.

Keywords: organic dairy farming, forage structure, milk yield from roughage

# Introduction

Driven by the increasing demand for organic dairy products in the last twenty years, organic dairy farming has developed rapidly, resulting in a wide range of production strategies which affect farm productivity (Haas *et al.*, 2007). In order to indentify forage cropping strategy types (FCST), we classified organic dairy farms by their particular structure of forage cropping areas and level of concentrate use. Based on this classification we analysed the variability of home-grown feedstuff quality and the milk yield from roughage (MYR).

# Materials and methods

The present investigation is based on a study that was conducted in 2007–2011 on 106 organic dairy farms in Germany, but utilizes only the data from 2008. These farms were visited by a team of advisers, who collected in questionnaire-guided interviews data on farm structure, feeding and forage management twice a year during the time of study. For the classification of FCST, four variables were determined: the share of permanent grassland, maize and forage grains, clover-grass and alfalfa, of the main roughage area of each farm. The amount of feeding concentrates (t cow<sup>-1</sup> year<sup>-1</sup>) was used as an indicator for intensity of energy feeding. Cluster analysis (Ward's method) identified six FCST (cophenetic correlation coefficient 0.79). Statistical analyses were performed using the Kruskal-Wallis test followed by multiple pair-wise comparisons of groups using the Wilcoxon rank sum test (adjusted with the Bonferroni-Holm correction).

Roughage sampling has been implemented after instruction from German Agricultural Analysis and Research Institute (LUFA Münster), the roughage analyses were performed

with Near-infrared spectroscopy (NIRS) according to the Association of German Agricultural Investigation and Research Institutions (VDLUFA) guidelines. Fat and protein corrected milk (FPCM) was calculated according GfE (2008). The MYR was calculated by a simplified equation, assuming an energy content of 7 MJ net energy for lactation (NEL) per kg concentrate. MYR [kg cow<sup>-1</sup> year<sup>-1</sup>] = FPCM [kg cow<sup>-1</sup> year<sup>-1</sup>] – (Energy from concentrates [kg cow<sup>-1</sup> year<sup>-1</sup>] ×7 MJ] / demand of energy for milk [3.28 MJ NEL kg<sup>-1</sup>]) Succulent feed or grass pellets could not be considered (fed on 10 of the investigated farms).

## Results

Cluster analysis revealed six different FCST (Table 1). Three permanent grassland-based types with 72% of all farms were found to be the majority, whereas the three forage cropping types were less frequent.

Tuble 1. Characteristics of Forage Cropping Strategy Types (FOST)									
FCST	CE	CI	CF	PE	PI	PM	D		
<i>n</i> farms	16	9	5	30	8	38	Г		
Median share of main roughage area:									
Permanent grassland %	50.6 <sup>b</sup>	40.2 <sup>ab</sup>	$20.4^{a(d)}$	97.1°	90.6 <sup>de</sup>	70.1°	0.000		
Maize and grain %	5.5 <sup>b</sup>	27.3ª	3.6b <sup>c</sup>	0.1°	4.7 <sup>b</sup>	7.8 <sup>b</sup>	0.000		
Grass-clover %	43.9ª.	32.5 <sup>ac</sup>	75.9 <sup>b</sup>	2.8 <sup>d</sup>	4.6 <sup>bd</sup>	22.2°	0.000		
Amount of concentrate t cow <sup>-1</sup> year <sup>-1</sup>	0.69ª	0.19°	1.04 <sup>abc</sup>	0.54ª	1.74 <sup>c</sup>	1.02 <sup>b</sup>	0.000		

Table 1. Characteristics of Forage Cropping Strategy Types (FCST)

This classification was further subdivided by the factor of intensity, determined by the average amount of concentrate application and the share of maize and forage grains. The following nomenclature of the six FCST was implemented: extensive and intensive cropping farming type (CE, CI), fodder cropping type (CF, with high share of clover grass), extensive and intensive permanent grassland type (PE, PI) and the mixed permanent grassland type (PM). The significantly highest MYR (P = 0.013) is recorded for PE (4700 kg, Table 2). In contrast, the intensive types (PI, CI) achieve only 3600 and 3700 kg MYR. Quality of maize silage and clover grass silage show no significant differences among the FCST (Table 2). Significant differences occur in feedstuffs from permanent grassland. The highest grass silage quality could be detected in PE and CI (Table 2). The highest forage value of hay is also to be found in PE (Table 2).

	2		0		,			
FCST		CE	CI	CF	PE	PI	PM	р
MYR (FCPM $cow^{-1} a^{-1}$ )	mean	4295 <sup>ab</sup>	3578ª	3830 <sup>ab</sup>	4711 <sup>b</sup>	3687 <sup>ab</sup>	4038 <sup>ab</sup>	0.013
	n	8	9	2	1	4	25	
Maize silage (MJ NEL kg <sup>-1</sup> DM)	mean	6.5	6.6	6.8	7	6.7	6.6	0.168
	n	10	7	2	19	8	28	
Grass silage (MJ NEL kg <sup>-1</sup> DM)	mean	5.7 <sup>ab</sup>	6.0 <sup>b</sup>	6.0 <sup>ab</sup>	6.1 <sup>b</sup>	5.7 <sup>ab</sup>	5.7ª	0.005
	n	11	6	4	4	1	22	
Clover-grass silage (MJ NEL kg <sup>-1</sup> DM)	mean	5.8	5.8	5.9	5.7	6.1	5.8	0.644
	п	12	1 5 2ab	3 5 1 ab	25	2	22	0.000
Hay (MJ NEL Kg <sup>-1</sup> DM)	mean	Э.Z"	5.3"	J.1™	⊃./°	4.6	5.0ª	0.000

Table 2. Quality of roughages and milk yield from roughage (MYR) in FCTS

## Discussion

In the present study permanent grassland is the major source of nutrition of organic-managed dairy herds in Germany (72% of 106 farms). Nevertheless, forage cropping-based systems can be found

as well. In both groups, intensity varies widely and so does herd size, milk yield and metabolic profiles and other factors (data not shown). Therefore one major conclusion of this investigation is that a tremendous variability of production figures exists among German organic dairy farms. This encouraged us to classify the farms leading to comparable conditions. In the analysis of MYR, significant group differences could be found among the six FCST (Kruskal-Wallis-Test, df = 5, P = 0.013; this is assumed to be in link to forage cropping structure modification. The variability of MYR within the FCST can be supposed to be management affected. The MYR is on a level similar to those calculated by Haas et al. (2007). Nevertheless, the intensive FCST (CI mean: 3579 kg MYR) do not achieve these MYR. The highest MYR is that of PE, which is due to excellent roughage quality of grass products and due to the low amount of concentrate use. Roughage qualities, especially those of grass products, vary within a wide range. The quality of maize silage (6.7 MJ NEL kg<sup>-1</sup> DM) is similar to that reported by Leisen et al. (2003) (6.5 MJ NEL kg<sup>-1</sup> DM), with a low level of variation. In contrast, grass silages of high quality can only be found in the FCST CI and PE. For PE it can be assumed that the excellent climatic conditions for forage production (data not shown) with precipitation of 1200 mm per year and intensive use promoting Lolium swards are the main reason for such good results. The poor quality of grass silage in PI has to be regarded as problematic, because low quality forces the farmers of PI to use high amounts of concentrate for fulfilling their milk quota, resulting in lower MYR. Furthermore, the quality of grass-clover silages is subject to a considerable variance (4.77–6.5 MJ NEL) with no clear relationship for the FCST. The best quality of hay is to be found in PE. where hay has a high importance, due to the use of milk for cheese production. For all roughage qualities, a positive correlation with MYR could be found (Table 3).

Table 3.	Pearson	correlation	coefficients	of ro	ughage	quality	and	milk	yield	from	rougha	ige
									~			

	Maize-MYR	Grass-MYR	Clover-Grass-MYR	Hay-MYR
Pearson correlations coefficient	0.18 ns	0.18(*)	0.55***	0.21*

This research clarified that FCST has an impact on MYR, and consequently on farm productivity (Scheringer, 2002). For the achievement of a high MYR, roughages of superior quality are required. If this requirement is not fulfilled, farmers tend to compensate with the use of concentrate – with consequences on the sustainability of the production (Scheringer, 2002). The possibility for achieving high roughage quality is partially affected by soil and climate conditions. Therefore, the production system has to be adapted to these conditions. Due to the lack of compensatory-possibilities, this constraint is even more important in organic agriculture than in conventional systems.

#### References

GfE (2008) New equations for predicting Metabolisable Energy of grass and maize products for ruminants. *Proceedings of the Society of Nutrition Physiology* 17, 191–204.

Haas G., Deittert C. and Köpke U. (2007) Impact of feeding pattern and feed purchase on area and cowrelated dairy performance of organic farms. *Livestock Science* 106, 132–144.

Leisen E. and Heimberg P. (2003) Milchviehhaltung. Schriftenreihe des Lehr- und Forschungsschwerpunktes Umweltverträgliche und Standortgerechte Landwirtschaft 105, 159–180.

Rougoor C.W., Vellinga T.V., Huirne R.B.M. and Kuipers A. (1999) Influence of grassland and feeding management on technical and economic results of dairy farms. *Netherlands Journal of Agricultural Science* 47, 135–151.

Scheringer J. (2002) Nitrogen on dairy farms: balances and efficiency. *Goettinger Agrarwissenschaftliche Beiträge* 10, Excelsior p.s., Goettingen.

# **Requirements for Decision Support Tools for grazing**

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# Abstract

Current trends in livestock farming are causing a decline in the popularity of grazing in Europe. Therefore, development and improvement of practical tools to facilitate grazing on dairy farms is stimulated. This study aims to assess the requirements for effective grazing Decision Support Tools in the Netherlands, since the currently available tools seem not to fit the needs of the farmers. As a first step we inventoried the specific needs of farmers in the Netherlands. As a second step we inventoried the available tools in Europe and beyond. Especially in Ireland, many farmers use decision support systems for grazing. These tools or adapted versions of these tools might be useful for other countries as well. We concluded that a grazing Decision Support Tool for dairy farmers in the Netherlands must be robust, simple and appealing. Predictability of the grazing system is a key factor. A simple dashboard tool could be developed which provides insight at a glance in the technical and economic consequences of management decisions.

Keywords: decision support, farmers, grazing, operational management, tool

# Introduction

Current trends in livestock farming as larger herds, introduction of automated milking systems (AMS), environmental aspects, but also uncertain factors as weather and difficulties with grassland management are causing a decline in the popularity of grazing in Europe. In the Netherlands, this is a matter of concern for the general public (Van den Pol-van Dasselaar et al., 2008). Currently the majority of farmers in the Netherlands practises grazing. In 2010, 74% of the Dutch dairy cattle were grazing at least part of the grazing season. In most situations, grazing has an economic advantage compared to no grazing (Van den Pol-van Dasselaar et al., 2010). However, farmers experience grazing as being difficult in several situations. Especially in situations of increasing herd size and or introduction of an automatic milking system (AMS), grassland can no longer be managed by historic knowledge or intuition. In those cases farmers lack the necessary skills to manage grazing properly. Therefore, development and improvement of practical tools to facilitate grazing on dairy farms is stimulated in the Netherlands. At the moment there are grazing tools available to facilitate Dutch farmers, but the farmers experience the available tools as complex or not suitable for practical use in their mixed systems with relatively high levels of supplementation. The tools provide general information and are not specific for individual farms. Therefore, the available tools are hardly used by farmers. Dairy systems in the Netherlands are rather intensive and many advisors do not advocate grazing at all.

This study aims to assess the requirements for effective grazing Decision Support Tools in the Netherlands, based on information in the Netherlands and surrounding countries. The latter information is important since there are several grazing tools available in Europe and beyond. The available tools may be adapted for the mixed systems with relatively high levels of supplementation of the Netherlands.

# Materials and methods

As a first step we inventoried the specific needs of farmers with respect to grazing in the Netherlands. We approached several farmers' networks (approximately 150 persons) and

advisors' networks (approximately 30 persons) and advertised in the media, asking for response from farmers and advisors. People were asked for their major concerns with respect to grazing, what type of information is needed to address these concerns, methods to facilitate grazing and wishes with respect to Decision Support Tools. Responses came from all regions of the Netherlands. Also, representatives of all farm types were included: intensive, extensive, small farms, large farms, farms with AMS, etc. As a second step we inventoried the available tools in Europe and beyond, using both literature and results from a survey toward the Working Group 'Grazing' of the European Grassland Federation and toward the International Farm Comparison Network (approximately 75 persons were approached in total). All results were analysed and discussed with a group of approximately 15 key stakeholders, including farmers, advisors and representatives of industry in the Netherlands.

## **Results and discussion**

The national survey showed that the main concerns of dairy farmers in the Netherlands with respect to grazing are:

- How to act on changing weather conditions which lead to reduced grass intake and declining milk production (weather changes quite often in the Netherlands).
- How to maintain a stable milk production under grazing (Dutch farmers are often aiming at high milk production levels, instead of aiming at a high profit or a high grass-use efficiency).

Staying in control is a big issue for the farmers and they feel unsure about their grazing management and the effect of external factors like the weather. When they are not in control it is difficult for them to see economic profits of grazing. Secondary concerns deal with peaks in labour and with difficulties related to increasing herd size and/or introduction of an AMS. Furthermore, Dutch farmers are unsure about their options to increase the grass intake and they are unsure about the grass yield available in the field. Finally, they are interested in information about the economics of grazing on a day-to-day basis.

Results from the international survey showed that in other countries in north-western Europe farmers have about the same concerns with respect to grazing as in the Netherlands. The international survey also showed that there are several tools available in Europe to support farmers (Table 1). The complex tools are used only by advisors. Farmers use basically their intuition and historical knowledge to manage their grasslands. Ireland with its pasture/grazing-based production is the exception to this rule. In Ireland, about 25% of the farmers measures grass, and the majority of the farmers uses grazing support tools in one way or another. Tools may be adapted for the mixed systems with relatively high levels of supplementation of the Netherlands.

Tool	Examples of countries where the tools are used
1001	(non-exhaustive)
Computer Decision Support Tools, sometimes	e.g. Ireland (feed wedge, grass budget planner),
web-based	France, Denmark
Decision Support Tools on paper	e.g. Luxemburg (Weideschieber)
Web-based forecast of grass production	e.g. France, Denmark
Grazing information platforms	e.g. Austria, Ireland
Tools to measure grass yield, e.g. grass height	Many countries. Rapid pasture meter is being
meter, rapid pasture meter	introduced from New Zealand to Europe

Table 1. Main tools for grassland management

The results show that the concerns of dairy farmers are related to:

- poor predictability of the grazing system (grass growth, herbage allowance, grass intake) and the effect of management on the grazing system.
- situations of 'forced' choices, i.e. grassland management must be adapted to changing circumstances and this has to be done now.

Based on the specific needs of the farmers in the Netherlands and the experiences in other countries, development of a grazing dashboard tool would be a good option ("GrazeTomTom" or "Grazing Navigation"). This was confirmed by the group of key stakeholders. The tool needs input on a yearly basis and a day-to-day basis. Annual inputs are, e.g. the number of parcels which can be used for grazing, surface area of parcels, number of animals and milk production. To provide results on a day-to-day basis, information on available DM yield, DM growth day<sup>-1</sup> and supplementation of the animals is needed. DM yield can be either measured or estimated. Measurement is rather complex and time consuming and will therefore not often be carried out by farmers. Simple and relatively labour-extensive measurement tools like the rapid pasture meter may lead to progress, but it will take some time before they will be introduced in practice in the Netherlands. In the meantime, DM yield can be estimated. DM growth day<sup>-1</sup> could be provided in newspapers and on the internet using average data for different regions of the Netherlands. Supplementation of the animals is usually well-known. The output of the dashboard tool could consist of two pointers: profit of grazing (accumulative starting January 1) and grass kg<sup>-1</sup> milk (or kg milk from grass). The dashboard tool could be available on internet or as an application for a smartphone. Furthermore, it could be supported by an extensive website with information and discussion groups on grazing management, economy of grazing etc. The website could also provide

## Conclusions

A grazing Decision Support Tool for dairy farmers in the Netherlands should be robust, simple and appealing. Predictability of the grazing system is a key factor. There are several tools available in Europe, which could be adapted for the mixed systems with relatively high levels of supplementation of the Netherlands. A simple dashboard tool, which provides insight at a glance in the technical and economic consequences of management decisions, could facilitate farmers in those common situations where they have to adapt quickly to changing circumstances.

more insight in the underlying mechanisms of the dashboard tool for those who are interested.

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## References

Van den Pol-van Dasselaar A., Vellinga T.V., Johansen A. and Kennedy E. (2008) To graze or not to graze, that's the question. *Grassland Science in Europe* 13, 706–716.

Van den Pol-van Dasselaar A., de Haan M., Evers A. and Philipsen A.P. (2010) Simulation of the effect of grass intake on the farmer's income. *Grassland Science in Europe* 15, 100–102.

# A quantification of phosphorus flows in The Netherlands through agriculture, industry and households

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# Abstract

Phosphorus (P) is a prerequisite for life. The natural resource for P fertilizer, rock phosphate, is a non-renewable resource and is concentrated in only a few countries in the world. The Netherlands have a large national P surplus and recycling is limited. To quantify all P flows and locate accumulation in the Netherlands, a study has started by assessing the year 2005. Analysis of the P flows in the Netherlands was performed by dividing the national system into a number of P-related subsystems: agriculture, industry, society, environment and waste handling. The national P balance of the Netherlands shows a large surplus, 59.7 Mkg P in 2005. The surplus accumulates in arable land (8.3 Mkg P), grassland + silage maize (23.0 Mkg P) and in waste, where a proportion is made unavailable (21.4 Mkg P). The loss to surface water is 6.8 Mkg P. The P in waste should be recycled. However, as long as animal manure is used and exceeds the output of agricultural land, recycling P from waste to Dutch agriculture will increase environmental problems. The P from waste and part of the animal manure should be processed and exported to regions where it is profitable and useful.

Keywords: phosphorus, phosphorus flow, national phosphorus flow, grassland, arable land

# Introduction

Phosphorus (P) is a prerequisite for life. Phosphorus is a major growth-limiting factor for plant production in the world. In 1850, P fertilizer was introduced into agriculture and its use has been increasing ever since. The natural resource for P fertilizer, rock phosphate, is a non-renewable resource and is concentrated in only a few countries in the world. Many western European countries, like the Netherlands, have a large national surplus and recycling is limited. Not only is a valuable resource wasted, but surface-water quality is deteriorating as a consequence of the accumulation of P in the soil. It is necessary to reduce the P surplus and increase recycling. Agriculture plays an important role in P flows in the Netherlands. The flows to and from agriculture are fairly well quantified. The soil P balance of agriculture is calculated annually by the Central Bureau of Statistics (CBS, 2007). The P flows in the non-agricultural part of the national system, e.g. industry and society, is not well quantified. To quantify all P flows and locate accumulation in the Netherlands, a study has started by assessing the year 2005. With the results of this study, it is possible to decide in which process most P accumulates in the country, and to decide where (more) recycling methods should be developed.

# Materials and methods

Analysis of the P flows in the Netherlands was performed by dividing the national system into a number of P-related subsystems (Figure 1): agriculture, industry, society, environment and waste handling. Agriculture includes grassland + silage maize, arable land, grazing animals

(cattle, goat and sheep), intensive animal husbandry (poultry, pigs, fur animals) and manure. Industry includes food, feed and non-food industry. Waste includes subsystems which are outside the scope of this paper. They are described in Smit *et al.* (2010).

The data that are necessary to estimate and calculate the P flows in the Netherlands, e.g. acreages and yields of crops, number of animals, animal production, use of fertilizer, import and export of food and feed components, were retrieved from national statistics (CBS, 2008). Animal manure was calculated by a balance method: intake minus production results in manure. Mineral- and organic-P fertilization was divided over grassland + silage maize based on data from the BIN-farm network (farm accountancy network; LEI, 2005). The data were processed in a Material Flow Analysis (MFA) which provides a systematic assessment of the flows and stocks of materials within a system defined in space and time (Brunner and Rechberger, 2004). In MFA the subsystems and flows were defined. Some subsystems were allowed to accumulate P (e.g. agricultural land) and from other subsystems (e.g. industry and households) the flows were defined as results from other flows and no accumulation was assumed. The P leaching from agricultural ground was used from the emission registration in the Netherlands (www.emissieregistratie.nl) and was not (yet) a function of surplus.

## **Results and discussion**

The results are given in million kg P in a scheme representing the Netherlands (Mkg P, Figure 1). The results show that the national P-balance of the Netherlands had a surplus of 59.7 Mkg P in 2005.

This surplus accumulated on land for arable crops (8.3 Mkg P, 12.5 kg P per ha), grassland and silage maize (23.0 Mkg P, 18.7 kg P per ha), through waste (21.4 Mkg P) and part was



Figure 1. P-flows in the Netherlands (the size of the flow is indicated in the ellipse in Mkg of P; accumulation of P is indicated where appropriate in the square boxes)

lost to surface water (6.8 Mkg P). The P in waste was not (directly) vulnerable to losses to surface or groundwater but it was made unavailable as a nutrient. Half was incinerated and used for other purposes such as foundation for roads, almost a quarter was incinerated and used as an ingredient for cement, and the rest was used as landfill. The total national manure production was 71.1 Mkg P. Grazing animals made the largest contribution to manure production, 43.9 Mkg P, and 9.3 Mkg P of this was directly returned to grassland during grazing. Two-thirds of the intake of grazing animals (39.1 Mkg P) originated from the grassland + silage maize. The intake of manufactured feed was 21.0 Mkg P and production in meat and dairy was 16.5 Mkg P. As manufactured feed intake was larger than the output of products, expressed in P, manure production of grazing animals exceeded the output of grassland by 4.8 Mkg P. Assuming all manure from grazing animals was applied on grassland + silage maize, 8.7 Mkg P of manure from intensive livestock and 11.7 Mkg P of fertilizer was applied on top of that. From grassland + silage maize, 2.1 Mkg P was lost to surface water. This resulted in 23.0 Mkg P of accumulation on grassland + silage maize. The total amount of P in animal manure exceeded the total output of agricultural land by 17.2 Mkg P. From the manure, 7.0 Mkg P was exported, and the import of fertilizer was 21.0 Mkg P. The surplus for all agricultural soil was 31.3 Mkg P (16.5 kg P per ha). In the near future, the accumulation on agricultural land will be limited by law: in 2013 a balanced fertilization (input = output) is required in the Netherlands.

To reduce the surplus in the national balance, P from waste should be recycled. However, due to the P in animal manure and accumulation in agricultural soils, recycling the P from waste to Dutch agriculture would enhance the environmental P problems and the surplus in the national balance would not be reduced. The excess of P should be exported, as long as livestock numbers are not reduced.

## Conclusions

The national P-balance of the Netherlands shows a large surplus, 59.7 Mkg P in 2005. The surplus accumulates on arable land (8.3 Mkg P), grassland + silage maize (23.0 Mkg P) and in waste from household and industry (21.4 Mkg P). The loss to surface water is 6.8 Mkg P. The P in waste is for the most part made unavailable as a nutrient because it is used as landfill, as foundation for roads and in cement. Since P is a non renewable resource, P in waste should be recycled. However, as long as the production of animal manure exceeds the output of agricultural land, recycling the P from waste to Dutch agriculture will increase environmental problems. The P from waste and a proportion of the animal manure should be processed and exported to regions where it is profitable and useful.

## References

Brunner P.H. and Rechberger H. (2004) *Practical Handbook of Material Flow Analysis. Advanced Methods in Resource and Waste Management*. Lewis Publishers, CRC Press Company, London, 318 pp. CBS (2008) Stroomschema's stikstof, fosfor en kalium 2004–2006, www.cbs.nl.

CBS (2007) Land- en tuinbouwcijfers 2007. LEI & CBS, 270 pp.

LEI (2005). BINternet, www.lei.wur.nl.

Smit A.L, Van Middelkoop J.C., van Dijk W., Van Reuler H., De Buck A.J. and Van De Sanden P.A.C.M. (2010) *A quantification of phosphorus flows in the Netherlands through Agricultural production, industrial processing and households*. Plant Research International, Wageningen, Report 364, 55 pp.

# Development of an evaluation tool to compare the performances of different cropping schemes to produce lignocellulosic biomass for energy

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# Abstract

Problems associated with energy supply and use of fossil fuels support the development of renewable energy. The use of lignocellulosic crops to generate bioenergy takes part to this dynamic. In this context, the INTERREG IV project "ENERBIOM" aims at identifying the best agricultural biomass sources pertaining to their transformation process either into biogas, second generation bioethanol or solid biofuel for combustion. An evaluation tool (ENVINECO) was developed to identify the most sustainable cropping modalities of different lignocellulosic crops from ecological and economic points of view. The energy cost was based on direct and indirect energy consumption: machinery fuel consumption and energy used to produce agricultural inputs. The economic cost took into account inputs and mechanization costs. The environmental cost was closely linked to the energy one. The model simulated the amount of greenhouse gases emitted by different technical operations. In this paper, we present this tool and its application on contrasted cropping modalities used to produce Festuca arundinacea Schreb. Compared modalities aimed at replacing some inputs, mainly N and herbicides, by more ecological alternatives: legume associations, fertilization scheme based on manure valorisation, mechanical or mixed weeding strategies. The results underlined the need to develop associations with legume species in order to reduce both energy needs and GHG emissions associated to plant biomass production.

Keywords: economic performances, environmental performances, GHG balance

# Introduction

Within the current energy context in the European Union, such as climate change, oil price peaks, insecure energy supply, and 2020 goals of the European Directive 2009/28/CE, agricultural biomass production could be considered as a potential renewable energy supply (Ghysel, 2010). In particular, biomass from grassland used for energy can contribute to sustainable development (Bühle, 2011). However, it is essential to identify the most sustainable ways to produce such biomass in order to optimise economic, environmental and energy returns.

To reach such a target, a decision support tool called ENVINECO was developed in the context of the ENERBIOM project (INTERREG IV program – Grande Région) in order to compare economic, greenhouse gases and energy balances of contrasted cropping schemes applied to different plant species potentially used to produce biomass for energy. The tool is presented in this paper together with its application on contrasted cropping modalities used to produce *Festuca arundinacea* Schreb.

# Materials and methods

In order to quantify ecological and economic performances of contrasted lignocellulosic crops and cropping systems, each modality has to be divided into its different cropping operations: ploughing, harrowing, sowing, weeding, fertilising and harvesting. For each

operation, machinery type, number of passages, input type and quantity of input need to be defined. Some of these elements can be chosen by the end-user of the decision support tool. In order to convert cropping operations into ecological impacts and economic cost, the following databases have been used: 'Mecacost' (Rabier and Misergue, 2008), 'Planete' (Bochu, 2007) and 'Ges'tim' (Gac et al., 2010). 'Mecacost' compiles energy consumption and economic costs linked to the use of a large panel of agricultural machinery. 'Planete' propose some coefficients to quantify the energy performances of farming systems. These coefficients were mobilised to quantify the energy performances of the cropping systems to be analysed. 'Ges'Tim' coefficients were used to quantify greenhouse gases (GHG) emissions linked to the different cropping schemes to be compared. The tool was implemented in Excel. In a first sheet, named 'scenario', the user determines his cropping modalities: fertilization scheme, quantity and type of plant protection product, etc. It allows adapting the tool for each cropping system and region. The sheet 'plants' includes the cropping modalities and the corresponding operations for the plant species studied in this project, while the last sheets integrate the mobilised conversion coefficients. Results were displayed in different graphs illustrating the relative importance of the different inputs and consumption items from the energy, economic and environmental points of view. Consumption items taken into account in this tool were linked to seeds, fertilisers, plant protection products and mechanisation inputs.

We illustrate hereafter the results obtained based on the the application of this evaluation tool on contrasted cropping modalities applied to *F. arundinacea*, a fodder crop with potential interest for energy biomass production, in a trial in 4 randomised blocks performed in Libramont (49°55'N, 05°24'E, 522 m a.s.l., Belgium). Two factors of variation were tested in this trial: fertilization and weeding. The 7 modalities compared were: chemical weeding with 0 (modality 1), 80 (2), 160 (3) or 240 (4) kg N ha<sup>-1</sup>; association with red clover and with 0 (modality 5) or 80 (6) kg N ha<sup>-1</sup>; mechanical weeding and 80 kg ha<sup>-1</sup> of organic N (7).

## **Results and discussion**

Figure 1 illustrates the results obtained while comparing the 7 cropping (chemical weeding with 0 (1), 80 (2), 160 (3) or 240 (4) kg N ha<sup>-1</sup>; association with red clover receiving 0 (5) or 80 (6) kg N ha<sup>-1</sup>; mechanical weeding with 80 kg ha<sup>-1</sup> of organic N (7)) schemes tested to produce *F. arundinacea.* Fertilization and, to a lesser extent, mechanisation, especially with mechanical weeding (weed-head mowing) and organic N fertilisation (modality 7), have the biggest impacts on energy consumption. An increase of mineral N supply led to a clear increase of indirect (energy necessary to produce and transport fertiliser) energy consumption. The modality 5, with red clover association, allowed to reach similar productions than modality 2 (chemical weeding with 80 kg N ha<sup>-1</sup>) while reducing energy consumption by three fold.

The low productions recorded under the modalities with red clover and mineral fertilisation (6) and with mechanical weeding and organic fertilisation (7) led to high production costs and the highest GHG emissions per t DM. This needs to be validated by additional observations, as organic fertiliser value increases from year to year through recurrent applications: long-term effect adding to the direct effect. As for energy consumption, the modality 5 allowed a two-fold reduction of GHG emissions in comparison with the modality 2.

These results illustrate how ENVINECO allows comparing, and thus identifying, the most sustainable ways, for producing energy crops by considering energy and economic costs together with greenhouse gases balance. Nevertheless, some environmental impacts were not taken into account in this tool (water quality, biodiversity, soil pollution...) as they usually are in Life Cycle Assessment (Halleux, 2008). However, classical Life Cycle Analysis does not integrate economic performance indicators as ENVINECO does.



Figure 1. Mean yields; direct and indirect energy needs; inputs and mechanisation costs; and GHG balances associated to the 7 cropping schemes tested to produce *F. arundinacea*. The 7 modalities were: chemical weeding with 0 (1), 80 (2), 160 (3) or 240 (4) kg N ha<sup>-1</sup>; association with red clover and with 0 (5) or 80 (6) kg N ha<sup>-1</sup>; mechanical weeding with 80 kg ha<sup>-1</sup> of organic N (7)

# Conclusions

As illustrated by these first results, this tool allows assessing the economic, energy and environmental 'costs' of different energy crops and cropping schemes in order to identify the best cropping pathway to produce sustainable and renewable energy from these crops. These results also underlined the need to develop the associations with legume species in order to reduce both energy needs and GHG emissions associated with plant biomass production. ENERBIOM also analyses the energy value in terms of biogas, bioethanol or heat production potential of the biomass obtained under the different schemes. Once these results are available, ENVINECO will allow the comparing of performances of cropping schemes by calculating (1) the net energy (MJ ha<sup>-1</sup>) linked to the biomass (useful energy less direct and indirect energy needed to produce this biomass), (2) the cost of this net energy ( $\mathbb{C} MJ^{-1}$ ) and (3) the GHG footprint of this net energy ( $\mathbb{C}O_2eq MJ^{-1}$ ).

## References

Bochu J.L. (2007) Synthèse des bilans Planete, Ademe, 30 pp.

Bühle L., Hensgen F. *et al.* (2011) Evaluation of fuel and biogas production on typical semi-natural grassland in Germany, Wales and Estonia. *Grassland Science in Europe* 16, 398–400.

Gac A., Le Gall A. *et al.* (2010) GES'TIM, guide méthodologique pour l'estimation des impacts des activités agricoles sur l'effet de serre. http://www.inst-elevage.asso.fr/spip.php?page=article\_espace&id\_espace=933&id\_article=17281.

Ghysel F., Godin B. *et al.* (2010) Valorisation énergétique des fourrages: Comparaison de trois filières, enjeux et opportunités. *Fourrages* 203, 163–174.

Halleux H., Lassaux S. *et al.* (2008) Comparative life cycle assessment of two biofuels. Ethanol from sugar beet and rapeseed methyl ester. *The International Journal of Life Cycle Assessment* 13(3), 184–190. Rabier F. and Miserque O. (2008) MECACOST, Un logiciel simple pour calculer le coût d'utilisation prévisionnel du matériel agricole. http://mecacost.cra.wallonie.be.

# Typology of specialized dairy farmers on the basis of their social engagement, grassland management rules and fodder stock constitution and valorisation practices

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# Abstract

Decisions taken by farmers to manage their fodder system are influenced by the economic, social, and environmental context which they are in line with. This work performed a typology of specialised dairy farms of Ardennes (Belgium) based on these decision rules. In order to identify these rules, open-ended interviews, exploring the diverse aspects of the fodder system (professional and private life targets, rules mobilised in grassland management and allocation to the different herds, in winter and summer diet definition,...), were carried out with 15 farmers. Based on this information, three groups of farmers were identified: "standard", "environmental" and "moderately environmental" dairy breeders. The main criteria to define these groups were: (1) the fertilisation scheme, (2) the first cut initiation and the fodder quality and (3) the composition of the summer and winter diet. The next step will be to discuss the impact of these different strategies on the feed autonomy and performance and therefore on the potential resilience of these farms.

Keywords: farming strategies, fodder stock constitution, fodder stock utilisation

# Introduction

Decisions taken by farmers to manage their fodder system are influenced by the economic, social, and environmental context which they are in line with. This has a clear impact on ruminant livestock farming system autonomy and therefore on its resilience in the context of increasing input prices. To explore this aspect, a typology of specialised dairy farms of Ardennes (Belgium) was performed based on these decision rules.

# Materials and methods

In order to identify the decision rules mobilised by dairy farmers of a northern area of Belgian Ardenne, open-ended interviews (Kaufmann, 2008) exploring the different aspects of the fodder system (rules mobilised in grassland management and fodder resources allocation to the different herds, in winter and summer diet definition...) were carried out, in February and March 2010, on 15 farms. The recorded interviews were integrally transcribed and inductively coded using computer-assisted qualitative data analysis software (Huang, 2009). Concepts evoked by breeders to describe the management of their systems (harvesting, conditioning and utilisation of their fodder resources with a special focus on grassland) were linked to interviews section(s). For each interviewed farmer, the presence/ absence of specific concepts for fodder production and utilisation (stocking rate, turn-in and turn-out dates, grazing management rules, milking yield, manure management, cutting dates and connected decision rules, harvesting mode...) were recorded. On this basis, contrasting management strategies were identified and described.

In order to cover the diversity of farming systems, a snowball sampling was used (Kaufmann, 2008). Every interviewed farmer was asked to name one or two colleagues with a feeding system that was as different as possible from their own system. The diversity observed in the group of farmers taking part to the study covers the diversity of the dairy systems in the study area as underlined in Table 1.

51 85				
System type	Proportion in the study	Proportion of the sampled		
System type	area	farms		
Mix Dairy and Beef Systems on grass with	160/	20%		
fodder crops (MC)	10%	20%		
Mix Dairy and Beef Systems on grass (M)	19%	20%		
Specialized Dairy Systems on grass with	2104	20%		
fodder crops (SDC)	2170	20%		
Specialized Dairy Systems on grass (SD)	45%	40%		

Table 1. Typology of dairy farms in the study area

## **Results and discussion**

The results of the open-ended interviews underlined the occurrence of some key observations, performed by all farmers involved in the project: (1) the first cut, a key point to manage vegetation development and for fodder stock production, (2) the interest for or against the use of a mixer-feeder wagon in accordance with the supplementation strategy and (3) the attention paid to stock breeding. The following parameters allowed us to discriminate three farming groups (Figure 1): (1) the farmer's targets in terms of production intensity, (2) the farmer's sensitivity to floristic criteria, and (3) fodder resource valorisation strategies (among others: fodder resources allocation to the different herds, in winter and summer diet definition). Three farmers out of the 15 interviewees run 'atypical' farming systems (farmer with two objectives like meat and milk/cattle breeding or farmer with an outsourcing of heifer breeding...) and were therefore not classified.

The "Standard" dairy farmers (= 25% of the interviewees) like to engage or at least participate in the social life of their community. They also develop some diverse pathways (tourism, direct marketing...) and they intensified their fodder production through high mineral and organic fertilisation rates. They pay special attention to the *graminae* component of the sward and they allocate their best fodder resources to their dairy herd. The average level of milk production of these herds was higher than 7500 litres per cow and is thus higher than the production level of the two other groups.

The "Environmental" dairy farmers (= 30% of the interviewees) like to participate in social events but seem to have less leisure time to organise themselves. They are more orientated towards an organic production mode and they apply only a limited amount of nitrogen on their fields in order to maintain the legume content in their swards. Successive cuts are usually stored in the same silo and are provided altogether to the different animal categories. The average level of milk production of these farms ranges from 6000 to 7000 litres per cow.

The "Moderately environmental" dairy farmers (= 25% of the interviewees) were less involved in the social life in their community. This observation could be linked to the huge workload derived from the management of two cattle herds, one for meat and one for milk production. These farmers pay special attention to the *graminae* component of their swards. They also apply a limited amount of nitrogen on their fields and allocate their best fodder resources to their dairy herd. The average level of milk production of this farm group ranged from 6500 to 7500 litres per cow.



Figure 1. Typology of specialised dairy farms based on their production targets, social engagement and grassland management and valorisation strategies

## Conclusions

The results allow a classification of dairy farmers on the basis of their social engagement, grassland management rules and fodder stock constitution and valorisation practices. The next step will be to analyse the impact of these strategies and practices on farm performance and resilience in a changing world context.

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# References

Huang R. (2009) RQDA: R-based Qualitative Data Analysis. Kaufmann J.C. (2008) *L'entretien compréhensif.* 2 éd. Paris, Armand Colin.

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