EGF at 50:
The Future of European Grasslands

Edited by

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EGF at 50:
The Future of European Grasslands

Proceedings of the 25th General Meeting
of the European Grassland Federation
Aberystwyth, Wales
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Foreword

The title of this, the 25th General Meeting of the European Grassland Federation, is 'EGF at 50: the future of European Grasslands'. Fifty years is an important milestone in the life of any organisation, so this theme encompasses an element of 'taking stock' as well as looking to the future. The EGF was set up in 1964 as a forum for research workers, advisors, teachers, farmers and policy makers with active interest in all aspects of grasslands in Europe. Its objectives are to facilitate and maintain close contact between grassland organizations in Europe, to promote the interchange of scientific and practical experience between grassland experts and to initiate conferences and other meetings on all aspects of grassland production and utilization in Europe. We hope that this conference will make a useful contribution to furthering the aims of the EGF. We have great pleasure in extending a warm welcome to all delegates to Aberystwyth, the location of this 25th General Meeting. Aberystwyth has been strongly associated with grassland agriculture, and in particular the breeding of forage and cereal crops, since the foundation of the Welsh Plant Breeding Station (WPBS) in 1919. The influential grassland scientist and environmentalist Sir George Stapledon was its first Director, a post he held from 1919 to 1942. Stapledon argued that grasslands were at the heart of successful agriculture, which in turn was at the heart of the UK's economic and spiritual well-being. For many years his vision was allied with the requirement to increase production from all types of grassland. One of the ways in which he had a direct impact on grassland production was through the development in WPBS of the 'S' varieties of grasses, clovers and oats. The highly successful S23 perennial ryegrass was launched in 1933 and S184 white clover was first marketed in 1942. Stapledon was a strong advocate of the use of grass-legume mixtures as leys, realising the importance of nitrogen transfer from legume to grass to cereal. Varieties such as the 'S' types enabled grassland to support higher levels of stocking, and therefore production per unit area. The political/social environment in which agriculture operates in the UK and other European countries has changed dramatically since Stapledon's time, and the very necessary focus on increasing productivity that prevailed during the early and middle twentieth century has now widened to incorporate environmental concerns. Overproduction has been checked by a number of political and economic drivers, and the emphasis is now firmly on sustainability.

It hardly needs to be stated nowadays that grassland fulfils a truly multifunctional purpose, supplying forage for animals, regulating water flows, storing carbon, preventing soil erosion, providing habitats for species in all trophic levels, and playing an important cultural role in society. But it could be argued that public awareness of the real value of grassland is still evolving, and there is much room for greater engagement of grassland science and scientists with wider society. The breadth of topics covered by the submissions to this conference shows that grassland scientists across Europe and beyond are actively engaging with grassland as a multifunctional entity, and research on all the issues mentioned above is represented by high quality posters and theatre papers. The opening session of the conference comprises three plenary papers providing an overview of European grassland research in Nordic, Temperate and Mediterranean regions. This is followed by plenary and submitted papers grouped into five Themes, plus one 'extra' Theme on forage crop improvement which was added to accommodate many high quality contributions on this topic.

We hope that EGF 2014 will be characterised by fruitful discussions and enjoyable social interactions between grassland scientists, farmers, advisors and sponsors. On behalf of the Organising Committee we express our sincere gratitude to the many people who have contributed to the success of this conference – the Executive Committee and Secretary of the EGF, the external reviewer, Alan Hopkins, Aberystwyth University Conference Office, the IBERS staff responsible for the many different organisational aspects, the farmers who hosted mid-conference tours, the British Grassland Society who organised the post-conference tour,

technicians and staff of the Arts Centre, entertainers at the various social events, official speakers at the opening ceremony and conference dinner, poster competition judges, and of course the conference session chairs, speakers (plenary and submitted) and poster presenters. We also thank our sponsors for their generous support. Financial input from the Stapledon Memorial Trust met the full cost of printing the conference Proceedings and sponsored the early-career scientist master classes, and the British Grassland Society sponsored the Opening Session and social event. We gratefully acknowledge this support. Finally, we thank you, the delegates, for attending this conference. We hope you will enjoy your time here and return to your countries with warm memories of Aberystwyth.

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President
European Grassland Federation

Vicky King
Secretary
Organising Committee

Rosemary Collins
Chair
Scientific Committee

Table of Contents

Theme 2 'Grasslands and ecosystem services'

Theme 2 invited papers

Functions of grassland and their potential in delivering ecosystem services

Theme 2 submitted papers

Theme 2 posters

Theme 4 'Livestock production'

Theme 4 invited papers

Theme 4 submitted papers

Theme 4 posters

Theme 5 'MultiSward'

Theme 5 invited papers

Theme 5 submitted papers

Theme 6 'Approaches to forage crop improvement'

Theme 6 submitted papers

Special paper

'The European Grassland Federation at 50: past, present and future'

The European Grassland Federation at 50: past, present and future

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Abstract

In 1963 the European Grassland Federation (EGF) was founded in the UK. The origin and membership over fifty years are described. The changes in grassland research and development are reflected in the subjects of the General Meetings and Symposia. The successful development in more recent years of Working Groups, Master Classes and Workshops is described. The links to the European Union and the future direction of the EGF are explored. The EGF continues to play an important role as a non-political forum for exchanging and communicating the results of grassland science in Europe and in bringing grassland scientists together. Future challenges for the EGF are discussed.

Keywords: grassland research, conferences, publications, education, European Union

Introduction

In the years following World War II, scientists contacted each other bilaterally at occasional European conferences and also at International Grassland Congresses. To improve contacts, in 1963 the European Grassland Federation (EGF) was officially established at a special Symposium at Hurley, UK, organized by the British Grassland Society. At this inaugural meeting, representatives in attendance from grassland societies and countries in Europe agreed to the formation of the EGF, adopted the constitution and appointed the first Executive Committee charged with organization of the first General Meeting in The Netherlands in 1965 (Powell *et al.*, 1995).

Since that time important changes in the European political scene have taken place, and these have affected the EGF. This article reviews important developments in EGF over 50 years, with particular attention to the years since 2003. The history of the first 40 years was described in full by Prins (2004) and is also available from the EGF website (*www.europeangrassland.org*).

EGF organization

The objectives of the EGF were and still are: to facilitate and maintain close contact between European grassland organizations, to promote the interchange of scientific and practical experience between grassland experts, and to initiate symposia and other meetings between European grassland organizations.

As was explained before (Prins, 2004), the EGF has a simple structure with membership open to national or representative grassland organizations in Europe. By 2013 thirty-one countries had become Full Members (Table 1). For those countries without a national or representative organization, individual grassland workers may become Corresponding Members to represent their country. In 2013 there were four corresponding members (Table 1). Membership of the EGF is gratis.

Contact between EGF and grassland specialists throughout Europe is maintained through country representatives who act as intermediaries between their national colleagues and the Federation Secretary. Europe has been divided into seven regions and each region is represented in the Executive Committee which manages the affairs of the Federation.

Table 1. Representation of European countries in seven regions: thirty-one full members and four corresponding members* as of 2013.

EGF region	Members and corresponding members*
Central Europe	Austria, Czech Republic, Germany, Hungary, Slovakia, Switzerland
Western Europe	Belgium, France, Ireland, The Netherlands, United Kingdom, Luxembourg*
Northern Europe	Denmark, Finland, Norway, Iceland, Sweden
North-eastern Europe	Estonia, Lithuania, Poland, Latvia*
Eastern Europe	Bulgaria, Romania, Russia, Ukraine
South-eastern Europe	Bosnia-Herzegovina, Croatia, Slovenia, Serbia, Macedonia*
Southern Europe	Greece, Italy, Portugal, Spain, Albania*

The EGF has Honorary Life Presidents who help to take care of continuity. Dr W. Davies, the great stimulator and founding father of the EGF, was voted the first Honorary Life President at the inaugural meeting in 1963 and he occupied that position until his death in 1968, playing an active role in the EGF (Powell *et al.*, 1995). Three more founding fathers, namely Prof. 't Hart, Dr. Järvi and Dr. Caputa were elected in 1980 and 1982 (Table 2). Not until 2000 were any further Honorary Life Presidents elected. To make the choice more objective, the Executive Committee decided that candidates should be able to show a set of achievements within EGF (Prins, 2004). Since then, seven more have been elected (Table 2). They are expected to attend meetings of the Executive Committee and to support and advise the Federation Secretary and Organizing Committees.

Table 2. Honorary Life Presidents since 1963.

Name	Country	Year elected	Year of death
Dr. W. Davies	UK	1963	1968
Prof. M.L. 't Hart	The Netherlands	1980	2005
Prof. N.G. Andreev	USSR	1980	1996
Dr. V. Järvi	Finland	1982	1987
Dr. J. Caputa	Switzerland	1982	1992
Dr. J. Frame	UK	2000	2006
Prof. G. Blagoveschensky	Russia	2000	
Prof. L. 't Mannetje	The Netherlands	2002	2008
Prof. R.J. Wilkins	UK	2002	
Prof. J. Nösberger	Switzerland	2004	
Dr. W.H. Prins	The Netherlands	2004	
Prof. J. Parente	Italy	2010	

Scientific Advisory Board

To assist the Executive Committee with strategy and focus on the right themes, subjects, authors and keynote speakers, a Scientific Advisory Board was established in 2012. The Board consists of independent senior researchers who are well known and actively involved with

grassland research. The Board does not meet, but is consulted by the Federation Secretary on an *ad hoc* basis. Presently the Board comprises some 13 experts.

The EGF Secretariat and Fund

In the early years EGF business was handled by the Secretary of the current Organizing Committee of the General Meeting. Thus the EGF secretariat changed hands every two years. This hampered the contact between member countries and within the Executive Committee. As from 1973, a voluntary continuing Federation Secretary was elected. Through the years this post was filled by Dr. R. Tayler (UK, 1973-1978), Dr. J.W. Minderhoud (The Netherlands, 1978-1985), Dr. W.H. Prins (The Netherlands, 1985-2004) and Dr. W. Kessler (Switzerland, since 2004).

With the growth of EGF, the decision to ensure a high and consistent standard in the level of EGF conferences and proceedings, as well as to promote EGF as European forum of grassland science, the task of the Federation Secretary has become more and more important. These days communication is mostly through e-mail, with general information distributed via the EGF website mentioned above. However, the Secretary has to ensure that important messages and documents are printed and kept for the archives of the Federation.

As country membership is free, secretarial costs of EGF administrative affairs had to be covered by conference budgets. In the early years this often meant that the costs were met by the Secretary's employer. However, in 1982 in Reading, a fund was founded so as to facilitate continuing activities of the EGF. Money became available from the financial budgets of EGF conferences via a levy of 10 Swiss Francs (SFr) per paying participant, as part of the registration fee. From the start of the EGF Fund, the Swiss Grassland Society (AGFF) has handled the fund, for which EGF is grateful.

The EGF Fund is supplemented by the net proceeds of sales of EGF Proceedings in the series 'Grassland Science in Europe', presently carried out through the good office of the Federation Secretary in Zürich.

General Meetings and Symposia

From the start it was decided to use only English for general communication at EGF conferences, both orally and in writing. The EGF can be proud to have opted for this unifying solution and is happy that native English-speaking colleagues are always prepared to assist with 'anglicising' the conference papers. These conferences are General Meetings lasting four days, intended for a wide group of grassland people, and Symposia of three days, for specialist topics. The General Meetings include scientific and social programmes and a Business Meeting. Table 3 lists the General Meetings since 1965. The scientific programmes of the first ten meetings generally concerned the production, quality, forage conservation and economics of native and sown grasslands as affected by climate, species, soil and nutrition. From the 11th General Meeting in 1986, the topics reflected the wider implications of grassland use, e.g. energy, sustainability, society, ecology, biodiversity and multi-functional grasslands.

At the first General Meeting in Wageningen some 100 participants were present. Through the years the number has increased and nowadays about 300–400 people are expected to attend the General Meetings. The record was 600 in La Rochelle (2002). Naturally, the majority originate from Europe, but participants from outside are welcomed and there are regular attendants from North America and Japan.

For specialist topics EGF invites member nations to organize symposia. In the early years these were occasional symposia, mostly dealing with conventional grassland farming. They featured mainly agronomic subjects (Hurley, 1963; Aberdeen, 1968; Wageningen, 1980 and 1987; Graz, 1991) or were more focused towards economics (Versailles, 1965) or forage conservation (Brighton, 1979; Braunschweig, 1991).

Table 3. EGF General Meetings, 1963–2013.

1st	Nitrogen and Grassland. Wageningen, The Netherlands. June-July 1965
2nd	Evaluation of grassland production. Versailles, France. May 1967
3rd	Conservation of grassland products. Braunschweig, Germany. June 1969
4th	Comparison between natural and artificial grassland. Lausanne, Switzerland. June 1971
5th	Quality of herbage. Uppsala, Sweden. June 1973
6th	Pasture and forage production in seasonally arid climates. Madrid, Spain. April 1975
7th	Constraints to grass growth and grassland output. Gent, Belgium. June 1978
8th	Forage production under marginal conditions. Zagreb, Yugoslavia. June 1980
9th	Efficient grassland farming. Reading, UK. September 1982
10th	Impact of climate on grass production and quality. Ås, Norway. June 1984
11th	Grassland – facing the energy crisis. Setubal, Portugal. May 1986
12th	Grassland and animal production, now and in the future. Dublin, Ireland. July 1988
13th	Soil–grassland–animal relationships. Banská Bystrica, Czechoslovakia. June 1990
14th	Sustainable production from grassland. Lahti, Finland. June 1992
15th	Grassland and society. Wageningen, The Netherlands. June 1994
16th	Grassland and land use systems. Grado, Italy. September 1996. *Grassland Science in Europe, Volume 1*
17th	Ecological aspects of grassland management. Debrecen, Hungary. May 1998. *Grassland Science in Europe, Volume 3*
18th	Grassland farming: balancing environmental and economic demands. Aalborg, Denmark. May 2000. Grassland *Science in Europe, Volume 5*
19th	Multi-function grasslands: quality forages, animal products and landscapes. La Rochelle, France. May 2002. *Grassland Science in Europe, Volume 7*
20th	Land use systems in grassland dominated regions. Luzern, Switzerland. June 2004. Grassland *Science in Europe, Volume 9*
21st	Sustainable grassland productivity. Badajoz, Spain. April 2006. *Grassland Science in Europe, Volume 11*
22nd	Biodiversity and animal feed; future challenges for grassland production. Uppsala, Sweden. June 2008. *Grassland Science in Europe, Volume 13*
23rd	Grassland in a changing world. Kiel, Germany. August-September 2010. *Grassland Science in Europe, Volume 15*
24th	Grassland – a European resource? Lublin, Poland. June 2012. *Grassland Science in Europe, Volume 17*

From 1997 onwards, EGF Symposia have been organized regularly in alternate years between General Meetings. Table 4 shows that, since then, less attention has been paid to conventional grassland production, with precedence given to subjects such as grassland biodiversity (Poland, 1997; Estonia, 2005), grassland and woody plants (Greece, 1999), organic grassland farming (Germany, 2001), effects on environment and economy (Bulgaria, 2003; Belgium, 2007; Austria, 2011; Iceland, 2013) and alternative functions (Czech Republic, 2009).
Generally about 150-200 people are expected to attend the symposia, and the record was 280 at the Brighton, UK symposium (1979). Many symposia are also attended by participants from outside Europe.

Table 4. EGF Symposia, 1963–2013.

Symposia	
1st	The agronomic evaluation of grassland. Hurley, UK. September 1963
2nd	Economic problems relating to the production of forages. Versailles, France. March 1965
3rd	Hill land productivity. Aberdeen, UK. June–July 1968
4th	Forage conservation in the '80s. Brighton, UK. November 1979
5th	The role of nitrogen in intensive grassland production. Wageningen, The Netherlands. August 1980
6th	Animal manures on grassland and fodder crops: fertilizer or waste? Wageningen, The Netherlands. August–September 1987
7th	Forage conservation towards 2000. Braunschweig, Germany. January 1991
8th	Grassland renovation and weed control in Europe. Graz, Austria. September 1991
9th	Management for grassland biodiversity. Lomza, Poland. May 1997. *Grassland Science in Europe, Volume 2*
10th	Grasslands and woody plants in Europe. Thessaloniki, Greece. May 1999. *Grassland Science in Europe, Volume 4*
11th	Organic grassland farming. Witzenhausen, Germany. July 2001. *Grassland Science in Europe, Volume 6*
12th	Optimal forage systems for animal production and the environment. Pleven, Bulgaria. May 2003. *Grassland Science in Europe, Volume 8*
13th	Integrating biodiversity and efficient grassland farming, Tartu, Estonia. August 2005. *Grassland Science in Europe, Volume 10*
14th	Permanent and temporary grassland: plant, environment, economy. Gent, Belgium. September 2007. *Grassland Science in Europe, Volume 12*
15th	Alternative functions of grassland. Brno, Czech Republic. September 2009. *Grassland Science in Europe, Volume 14*
16th	Grassland farming and land management systems in mountainous regions. Gumpenstein, Austria. August 2011. *Grassland Science in Europe, Volume 16*
17th	The role of grasslands in a green future – threats and perspectives in less favoured areas, Iceland. June 2013. *Grassland Science in Europe, Volume 18*

Events for young scientists, special workshops and project meetings

Since 2002, EGF General Meetings regularly include special offers, such as seminars or master classes, for scientists younger than 35 years, active in research, resident of an EGF country, with an accepted contribution for the conference and thus eligible for a reduced registration fee.

Moreover special workshops organized outside the scientific programmes of EGF conferences provide the opportunity to discuss subjects between researchers. Sometimes policy makers from the EU have participated in these workshops. Examples are the recent workshops held in Poland (2012) on: i) Grassland Production in Europe, about the information needs of Eurostat as well as the MultiSward Book Project, and on ii) EGF Resolutions on the Common Agricultural Policy (CAP) Reform of 12 October 2011.

The annual EGF conferences are also on offer, and have been used intensively for years, for project management and project team meetings of European research projects of various programmes (Interreg, COST, EU Seventh Framework Programme for Research FP7, EU Marie Curie Actions *etc*.).

These additional events connected to EGF conferences have proved to be very useful for the attendants, not only for technical reasons, but also for social reasons by extending and strengthening their networks.

From Conference Proceedings to the series Grassland Science in Europe

The Proceedings of General Meetings and Symposia have all appeared in print. At first each Organizing Committee took care of its own proceedings and was thus influenced by local editing and printing procedures. In the 1990s it was decided to opt for more standardization. All papers were read and checked by at least two referees and native English-speaking colleagues started to help with 'anglicising' the manuscripts. The final standardization took place with the proceedings appearing as consecutive volumes in the series 'Grassland Science in Europe' for both General Meetings (Table 3) and Symposia (Table 4). For 2014 it is already Volume 19 in this series. Discussions are taking place as to whether or not EGF should continue to produce paper copies of the Proceedings. These have been available on compact disc and on the EGF website since 2004.
As mentioned above, EGF welcomes participants from outside Europe to attend the conferences. However, papers from outside are only accepted if at least one author is from Europe and the work is relevant to Europe.

Grass and Forage Science, the official journal of EGF

In 1996 the British Grassland Society (BGS) and EGF joined hands in the publication of *Grass and Forage Science*. This cooperation, with the financial responsibility held by BGS, has contributed to the journal becoming much more international in its scope and focus. In the 1990s, papers submitted to *Grass and Forage Science* were predominantly from English-speaking countries, with very few from elsewhere in Europe. By 2013, around one-third of papers published in *Grass and Forage Science* were from authors based in mainland Europe. Many European countries are also now represented in the journal's Advisory Editors and Associate Editors. The journal has also increased its presence and attractiveness to authors and readers in other grassland areas of the world, notably China and Latin America.
Publication of the review paper on international grazing terminology in March 2011 was an important milestone in the journal's increasingly international appeal. Other important review papers, including some that have been adapted from plenary papers presented at recent EGF General Meetings and Symposia, have also contributed to the journal receiving increased citation metrics and downloads from the journal website. The EGF is grateful to BGS for taking care of the management of *Grass and Forage Science.*

Working Groups

In the early years EGF had already set up selected committees on specialist topics like 'Methods of Forage Production Experiments' or 'Nomenclature'.
Since 2002 EGF has acted as an umbrella for Working Groups of scientists from different European countries. These groups are welcome to organize their own meetings at EGF conferences and report at each EGF General Meeting. Recently they have started to report also on the EGF website. There are four active groups and one group has finished its work. The groups are listed below:

- Grassland re-sowing and grass-arable rotations (2002-)

- Dairy farming systems (2004-)

- Analytical methods for measuring fatty acids in forage (2006-2010)

- Grazing (2008-)

- Semi-natural grasslands (2011-).

More details can be found at the EGF website. The modern EGF conference provides a good forum for the encouragement of these and other multi-national and multi-disciplinary projects and for reporting their progress. This contrasts with the early days of EGF, when most papers reported the results of work carried out in one institute in one country and there were no working groups.

European Union (EU)

The EGF is the only European-wide non-political body that organizes regular meetings to discuss scientific and socioeconomic issues related to European grassland science, management and farming in the broadest sense. The Federation realized that it was in a position to provide expert information required for the evolution of EU policies, whilst the decisions of the EU would affect the adoption of technologies being developed by EGF scientists.

Efforts have been made over 20 years to increase contact between EGF and the EU. All General Meetings since 2002 have had plenary papers concerning the effects of EU policies on grassland. EU-officials came to EGF conferences and notably in 2008 the session on 'Grasslands – Challenge for the Future' included an address by the EU Commissioner for Agriculture and Rural Development.

Exchanges between officials of EGF and the EU have occurred since 1997, but it was not until 2011 that any continuing relationships developed. It was agreed with EU officials that workshop sessions would be held at the General Meeting in Poland in 2012 with EU participation and that the Federation would supply input to the debate on evolving EU policies for grassland. A Working Group met in Lublin and produced a series of resolutions that were adopted at the Business Meeting and submitted to the EU in July 2012.

Following this, the EGF was asked to provide advice to Eurostat on grassland. Prof. A. Peeters led a Working Group that submitted to Eurostat late in 2013 a paper entitled 'Grassland Term Definitions and Classifications Adapted to the Diversity of European Grassland-Based Systems'. In another significant development in October 2012, the EU invited EGF to nominate a representative on the Steering Board for the European Innovation Partnership on Agricultural Productivity and Sustainability (EIP-AGRI). After that, the Federation Secretary and Prof. A. Peeters were decisively involved in the EU's development of the Strategic Implementation Plan for EIP-AGRI.

It is too early to identify policy decisions that have arisen directly from inputs from EGF, but contacts are now well established and should enable more rapid and effective use of findings from our scientists in the evolution of EU policies.

In addition to these contacts at the policy level, EGF plays an important part in the execution and reporting of multi-national research programmes funded by the EU. The personal relationships between scientists at EGF meetings have been important in developing consortia to bid for and undertake these programmes. Increasingly, not only are early results reported at EGF meetings, but also working meetings of consortia are often attached to EGF meetings.

The future: challenges and perspectives

The paper on EGF History written ten years ago (Prins, 2004) included a number of statements on 'The Future'. We now comment on the extent to which our aspirations in these areas have been realized. Some new challenges are added.

1. 'Grasslands are now used increasingly for multi-functional purposes rather than solely for animal production. Increasing involvement of scientists from basic, social and environmental sciences is therefore to be expected'.

In the past ten years, indeed more participation from non-agronomic scientists took place in the conferences, which was, of course, related to the conference themes. Certainly more

speakers from outside the agronomy scene were invited to present a plenary paper. There is an increasing interest in how to meet the challenges of the various services which grassland provides for the human well-being and for its contribution to natural capital and environmental services. Furthermore, the evident atmospheric changes require new science-based information on how to support the preparedness of agriculture for climate change.

For the future, the effort by EGF to reach scientists outside the regular agronomy world should be increased, the more so because some renowned grassland research institutes have been substantially reduced or even closed and some university chairs of grassland science have been discontinued. New grassland-relevant scientific input has originated in institutes of ecology and other scientific societies offer platforms for the discussion of grassland issues, but a professional link to the agricultural relevance is often missing. The actual scientific environment for grassland science has substantially expanded; it requires strong collaborations among many disciplines, including agronomists, animal scientists, soil scientists, ecologists, economists, sociologists and modellers. Closer cooperation, both between scientists in different disciplines and between different Scientific Societies, would produce 'win-win' situations.

2. 'In order to provide emphasis on new and important areas, and to facilitate in-depth studies, the EGF encourages the formation of Special Interest Groups. There are many advantages of such Groups convening during or around EGF conferences.' The section above on Working Groups shows that good progress has been made in this initiative.

For the future, this effort should be maintained but EGF should try to involve more scientists from outside the disciplines that represented the previous core of EGF, as mentioned under 1.

3. 'The EGF is the only European-wide non-political body that organizes regular meetings to discuss scientific and socioeconomic issues related to European grassland science, management and farming in the broadest sense. In order to spread the social and political impact of the knowledge and understanding of grassland matter, the Secretariat of the EGF plans to issue press statements to the general and farming media in countries in Europe, before and after meetings.'

The planned press statements have not appeared regularly; the EGF, run by volunteers, should find a way to get this organized properly. A success is that, since 2009, at the end of every conference a senior scientist summarizes the results and this Synthesis then appears on the EGF website.

The following are new statements regarding challenges and perspectives:

4. There is a growing tendency to base important long-term policy decisions in Europe on results of short-term research. The EGF should send a wake-up call that these decisions can be dangerous and often misleading. The EGF stresses the need for long-term experiments in strategic agro-ecological systems. In the past those experiments repeatedly demonstrated that long-term responses differed markedly from short-term responses.

5. With the increasing world population and use of agricultural products for other purposes than food or feed, European politics may require that farmers should become more autonomous in the supply of feed, particularly protein, for the livestock sector. As a consequence grassland farming may be confronted with complex challenges which have to be solved by agricultural and environmental scientists together with the social science communities.

6. There is concern that the education of future grassland experts should be improved in most European countries. In this, EGF may be able to contribute by facilitating networking and exchange of information, not only via the usual conferences, working groups, master classes and workshops, but also by facilitating internships, mentors and career resources for the mutual benefit of early-career scientists, experts and interested institutions. Such actions will certainly support the efforts of the EGF to be perceived as the voice of grassland issues in Europe.

For decades EGF has played an integral role in advancing grassland research and management. It continues to anticipate new challenges and is prepared to address emerging issues in a manner

that is science-based, comprehensive and socially acceptable. The update of the core values of the Federation will be expressed in a new mission statement of EGF that is being drafted in consultation with the Scientific Advisory Board.

Acknowledgements

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European grasslands overviews invited papers

European grasslands overview: Nordic region

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Abstract

The Nordic countries stretch over a large geographic area and, hence, conditions for plant growth vary considerably across the region. The western regions along the North Atlantic Ocean enjoy a mild maritime climate whereas continental climate prevails in the eastern part. Temperature variations have implications for the length of the growing season and Accumulated Day Degrees, thus influencing the timing and number of harvests in different areas across the region. Grasslands in the Nordic region are a diverse group and their relative importance differs greatly across countries. Thus, natural grasslands are extensive in Iceland and play an important role for livestock production, whereas they are much less significant in the other countries. Semi-natural grasslands are used for grazing but they are also important for maintaining biodiversity and landscape types. Forage for winter fodder and dairy in summer is obtained from cultivated grasslands of which short-term leys dominate in the southern regions. Milk and beef is the dominant produce, particularly in Denmark, whereas lamb and horse meat is a significant part of the production in Iceland. The expected climate change at northern latitudes will result in a longer growing season and higher temperatures during the growing season, both of which may lead to increased biomass production potential. However, new types of stresses may offset the potential gain. This will have various implications for adapting forage cultivars to the changing conditions both through breeding and different management schemes.

Keywords: agricultural production, climate, forage mixtures, local adaptation, soil

Geography and the natural environment

The Nordic region consists of the Nordic countries, the Faroe Islands, Greenland and Åland. The present paper will primarily focus on grasslands in the five Nordic countries: Denmark, Finland, Iceland, Norway and Sweden. These countries stretch over a large geographic area from 54° 58' N in the south of Denmark to 71° 08' N in Northern Norway, and from 24° 32' W in the west of Iceland to 31° 35' E on the eastern border of Finland with Russia (Figure 1). There are currently around 25 million inhabitants, making the region one of the most sparsely populated parts of the world. Iceland thus has around 3 inhabitants per km^{-2}, Sweden, Norway and Finland each have 16–23 inhabitants km^{-2} whereas Denmark has 130 inhabitants km^{-2}, a value close to the EU mean.

Climatic conditions

Conditions for plant growth vary considerably across the region, reflecting its wide geographic spread. The Gulf Stream brings warm sea from the Gulf of Mexico into the North Atlantic Ocean resulting in a milder climate than could otherwise be expected from the geographic location. However, various factors can affect the temperature as indicated for example by the

10 °C isotherm line in July, which falls below the Arctic Circle in Iceland but stretches well beyond in Northern Scandinavia (Figure 1). The difference in daily mean temperature during the warmest period in summer between the warmest (e.g. Helsinki, FI) and coldest agricultural areas (e.g. Akureyri, IS) is thus around 7 °C and temperature can vary up to 5 °C between coastal areas (Reykjavík, IS) and inland (Kajaani, FI) at the same latitude during summer (Figure 2). Temperature variations have implications for the length of the growing season and Accumulated Day Degrees, thus influencing the timing and number of harvests in different areas across the region. The growing season can be defined as the number of days from when the daily mean temperature exceeds 5 °C in spring and falls below 5 °C in autumn (SNP, 1992). The length of the growing season varies thus from around 230 days in the south west, to around 125 days in the northern-most and high altitude regions where agriculture is at its limit (Table 1). Similarly Accumulated Day Degrees (Tsum >5 °C) are currently only around 640 in Reykjavík (IS) compared to 1732 in Tranebjerg (DK), and 1020 – 1150 at Værnes (NO), Holmögadd (SE) and Kajaani (FI), which are at comparable latitudes to Reykjavík.

Figure 1. The Nordic region showing the Arctic Circle (broken line) and the 10 °C isotherm for July (solid line). Letters show geographic locations of sites listed in Table 1.

Temperature conditions during autumn and winter vary similarly across the region, both from north to south and from coast to inland at the same latitude (Figure 2). In the more maritime regions winters are often relatively mild with mean temperatures during the coldest month varying from around 2 °C in Sola (NO) and Tranebjerg (DK) to -3.5 °C in Tromsø (NO). Here, winters are characterised by unstable snow cover and frequent freeze-thaw cycles. In the more continental regions to the east of the Norwegian mountain range temperatures during winter are, on the other hand, much lower ranging e.g. from around -5 °C in Helsinki (FI) to -13 °C in Sodakylae (FI) resulting in much more stable winters. Such varying conditions during winter have different implications for winter damages of grasslands across the region.

Soil conditions

Most soil types in the Nordic countries are formed on glacial materials from the last ice age (European Commission, 2005). Large areas of Sweden and Finland are covered by a continuous layer of glacial till dominated by podzols but these tend to be weakly developed in the northernmost parts. In Norway, on the other hand, the glacial till is thin and discontinuous, exposing large areas of bare rock, and varying both in texture and nutrient value. Smaller areas of sandy glacio-fluvial deposits can be found in all three countries. In the low-lying coastal areas along the Gulf of Bothnia silty and clayey deposits are common, while marine and river deposits around fjords and in valley bottoms are most important parent materials for the agricultural soils in the middle and southern parts of Norway. Peat covers around 15% of the area in Finland and Sweden, while in Norway peat soils constitute a considerable part of agricultural land in the western and northern part, all of which is used for grassland. Peat soils are acidic, of low nutrient value and have to be drained. Land use in Sweden is dominated by forestry (56% of land) while agriculture covers approximately 7% of the area. The best soils are mainly used for growing cereals and oil crops, while grassland production most often occurs on the better soils in forested areas, or poorer soils in otherwise crop dominated areas. In Denmark Podzol forms a continuous block over nearly the whole western half of Jutland. The sandy Podzols in the far west are unfertile and limit agricultural activity unless heavily fertilized and limed. These regions were almost all covered by heath until the beginning of the 20th century. In the eastern part of the country Haplic Luvisols, the most productive soils, predominate. Dairy farms are primarily placed on loamy-sand or coarse sandy soil where the plant available water is around 90 and 60 mm, respectively. Therefore most of the intensive managed grasslands on sandy soils are irrigated at drought stress. Soils in Iceland originate from parent material of recent volcanic origin, which consists mostly of basaltic tephra, and are classified as Andosols (Arnalds, 2004). Such soils generally contain a range of pore sizes that can retain large amounts of water. They are high in organic C and N, and have a strong tendency to fix phosphorus. All these characteristics provide an excellent environment for root growth (Nanzyo *et al*., 1993).

The role of grasslands in agricultural production and environmental protection

Extent and importance of different types of grassland

Grasslands in the Nordic region are a diverse group that can be classified in various ways depending on their origin, vegetation type and/or current land use. In this paper we will primarily focus on grasslands that play a role in animal husbandry. We will follow the definition of grassland as a term that 'bridges pastureland and rangeland and may be either a natural or an imposed ecosystem. The vegetation of grassland in this context is broadly interpreted to include grasses, legumes and other forbs, and at times woody species may be present' (Allen *et al*., 2011). In our discussion we will further divide grassland into three major types with respect to origin (Hejcman *et al*., 2012): (i) natural grasslands dominated by indigenous grasses and other herbaceous species; (ii) semi-natural grasslands created by long-term human intervention and with a wide range of species richness and herbage productivity; and iii) improved grasslands established with domesticated forage species that receive intensive management (fertilization, weed control, renovation).
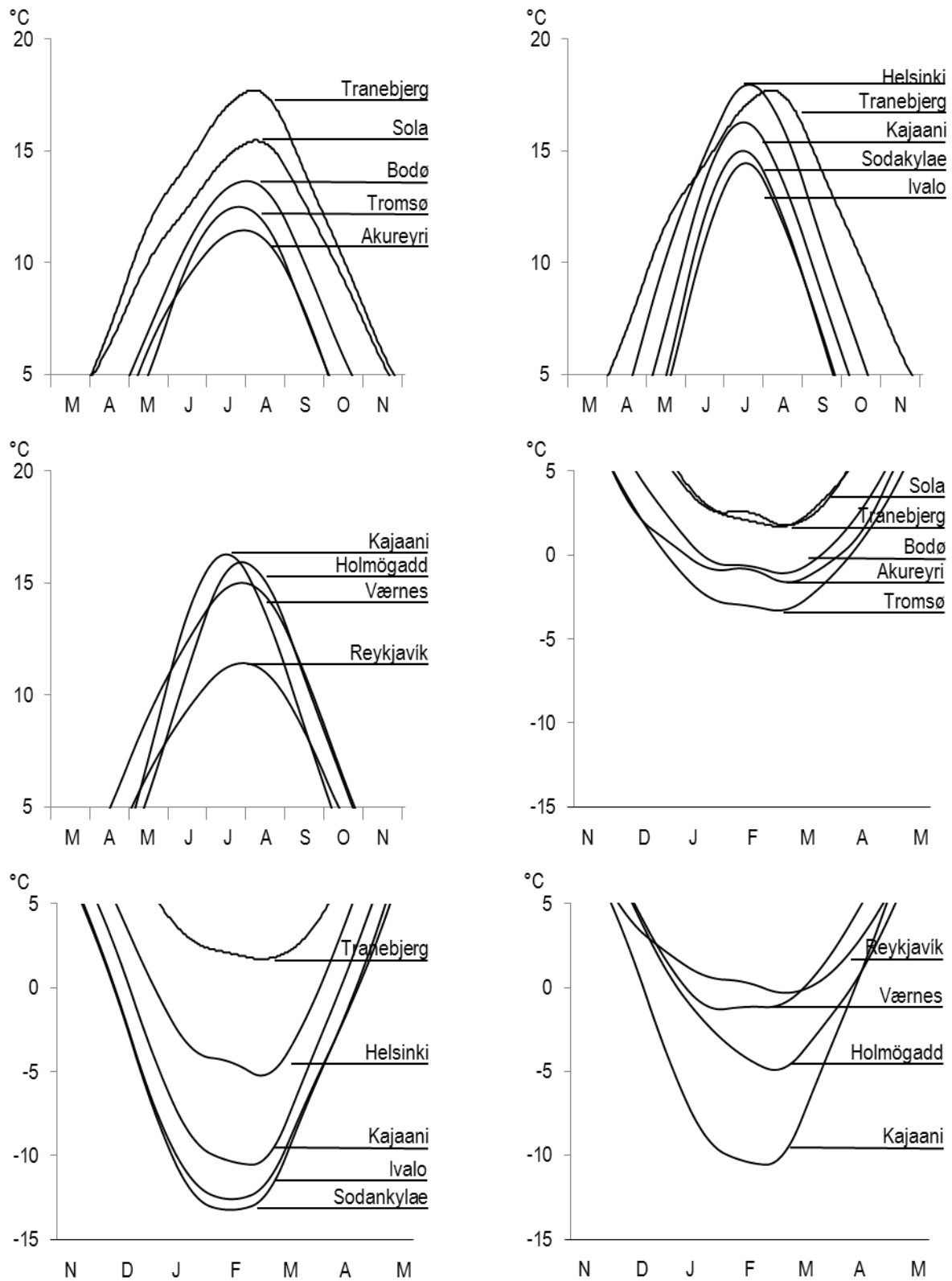
Figure 2. Estimates of mean temperature for each day of the year for the period 1986-2005 during summer (T>5 °C) and winter (T<5 °C) for selected sites in the Nordic countries, going from south to north in the maritime West (left) and continental East (centre), and from West to East at the same latitute of around 64°N (right) (for information of geographic location, see Table 1 and Figure 1) (estimates based on an improved method from Björnsson *et al.*, 2007).

Table 1. Geographic location, length of the growing season (T>5 °C) and Accumulated Day Degrees (° D; Tsum>5 °C) for selected sites in theNordic countries, based on estimates of mean temperature for each day of the year for the period 1986-2005 (Björnsson *et al*., 2007).

| | Site | Latitude | Longitude | Growing season | | |
				Period	No. of days	°D
A	Akureyri (IS)	65.7° N	18.1° W	9 May – 1 Oct.	146	669
B	Bodø (NO)	67.3° N	14.4° E	2 May – 19 Oct.	171	900
C	Helsinki (FI)	60.3° N	25.0° E	21 April – 18 Oct.	181	1379
D	Holmögadd (SE)	63.6° N	28.8° E	13 May – 20 Oct.	161	1028
E	Ivalo (FI)	68.4° N	27.3° E	21 May – 22 Sept.	125	718
F	Kajaani (FI)	64.3° N	27.7° E	6 May – 3 Oct.	150	1020
G	Reykjavík (IS)	64.1° N	21.9° W	4 May – 9 Oct.	159	642
H	Sodankylae (FI)	67.4° N	26.7° E	17 May – 21 Sept.	128	785
I	Sola (NO)	58.5° N	05.3° E	3 April – 17 Nov.	229	1391
J	Tranebjerg (DK)	55.9° N	10.6° E	2 April – 21 Nov.	234	1732
K	Tromsø (NO)	69.7° N	18.9° E	16 May – 1 Oct.	139	659
L	Værnes (NO)	63.5° N	12.9° E	17 April – 21 Oct.	188	1145

Natural grasslands

Virtually all of Icelandic grasslands are either rangeland or pastureland and therefore important for livestock production. There are discrepancies as to how grassland in Iceland has been defined. In connection with the land-use related GHG emission reported to the UN-Framework Convention on Climate Change, the Icelandic land-use category Grassland is defined as all land with > 20% coverage of vascular plants, and not included under categories Forest land, Cropland, Wetland or Settlement, and estimated to be 53,000 km^2 or 51% of the country (Gudmundsson *et al*., 2013). On the other hand, according to results obtained from the Icelandic Farmland Database, which classifies the surface of the country into twelve different classes based on satellite images and field research (Arnalds and Barkarson, 2003), only around 43,000 km^2 or 42% of the country is vegetated (Arnalds, 2011). In the present context two of these classes could be truly classified as natural grassland; 'grassland' being 2,375 km^2 or 2.3% of the country and 'rich heath' 6,843 km^2 or 6.6% or a total of 9,218 km^2 (Table 2).

Table 2. The extent of different types of grasslands (in thousand hectares) in the Nordic region.

	UAA[1]	Cultivated grasslands	Semi-natural grasslands	Natural grasslands
Denmark	2,645	332	200	0
Finland	2,285	650	40	0
Iceland	130	120	42	922
Norway	990	470	177	?
Sweden	3,030	1,100	440	0

[1] Utilizable Agricultural Area

These areas are characterized by low-growing vegetation of grasses, sedges, forbs and woody perennials. They are the most valuable part of the rangelands and used for extensive summer grazing of sheep and horses from the end of June to early September when the animals are rounded up. Around 40% of these natural grasslands are found above 200 m and each local community has grazing rights in the common grazing areas of the highlands. Roughly half of the required feed for sheep and a large part of feed for horses is obtained from summer grazing on the natural grassland.

The grazing resources of outlying fields in Norway are significant and these are currently being surveyed. Preliminary data for a grazing period of 100 days year^{-1} show that about 760,000 FEm (milk feeding units) may be utilized (Rekdal, 2013). Natural grassland rarely occurs in Sweden, Finland and Denmark.

Semi-natural grasslands

The Icelandic Farmland Database defines 'cultivated land' as being 1,723 km^2 or 1.7% of the country (Arnalds, 2011). Of this it is estimated that around 42,000 ha are 'abandoned crop land' (Gudmundsson *et al.*, 2013). Most if not all of this consists of old hayfields that have been taken out of use, mostly because of structural changes in agricultural production over the last few decades. In the present context these old grass fields can be defined as semi-natural grasslands in Iceland (Table 2). Some of these have been used for hay making over centuries but mostly they originate from the cultivation era that started around 100 years ago (Helgadóttir *et al.*, 2013). Even though most of the fields were originally sown with improved grass cultivars they are now dominated by indigenous species. Such ecotypes may become valuable for future breeding (Rognli *et al.*, 2013a).

In Norway grasslands are defined as areas covered with grass that may be mechanically harvested or grazed but never ploughed and that may be cultivated by fertilization, harvested mechanically and improved by selected species (CPA, 2013). Grazing lands are assigned to areas with at least 50% grass cover and annual grazing. Trees, stumps, and rocks may be present but grazing is considered the most important land use form either as surface-cultivated grass leys or unimproved grazing land (NFLI, 2011). Statistics Norway (2014) further defines the two types of grassland management: (1) surface-cultivated pastures have shallow topsoil layers, often with surface rocks, and can be mechanically harvested but are not ploughed, and (2) unimproved grazing land is never mechanically harvested (or ploughed) but only grazed and can be considered semi-natural landscapes. For the second class at least half of the area should be covered by grasses or palatable herbs and enclosed by a fence or a natural barrier. It must also be grazed or harvested at least once a year to be eligible for subsidy support (Kynding Borgen and Hylen, 2013). Such areas will also include active summer farms that still can be found in specific regions. In 2012 semi-natural grasslands amounted to 177,000 ha of which 20,000 ha were surface-cultivated pastures and 156,000 ha were unimproved grazing land (Statistics Norway, 2014) (Table 2).

In the past, Sweden had large areas of semi-natural grasslands, culminating at around 1.6 million ha at the end of the 18th century (Statistics Sweden, 2013), that were mainly used to provide winter fodder. Summer grazing took place in the forests. Today, only some 440,000 ha of these semi-natural grasslands remain, almost all of which are now used for grazing (Table 2). They play an extremely important role for the protection of biodiversity in the Swedish landscape, and farmers can receive subsidies if they are managed according to a set of rules aimed at the preservation of biodiversity (no fertilization, specified grazing regimes). The output in terms of animal produce is low but the marketing of beef to a premium price from cattle that have grazed in these areas is increasing.

In Denmark semi-natural grasslands cover 200,000 ha and are either not ploughed at all or only rarely. Some of these are on wet organic or sandy soils along rivers, near the coastline or on old hilly grasslands. This is therefore a very heterogeneous group with respect to yield, species composition and use. The aim of the authorities is to preserve these permanent grasslands in order to enhance a landscape of light and open (non-forest) river valleys and to maintain biodiversity. Strict rules apply to the management of these grasslands. Semi-natural grasslands are of minor significance in Finland and most of these can be found in coastal areas where the aim is to keep the landscape open to encourage bird life and natural meadows (Table 2).

Cultivated grasslands

The total area of cultivated land in Iceland in current use is around 130,000 ha (Table 2), of which approximately 45% is on drained wetland (Hallsdóttir *et al.*, 2012). Around 90% of this area is used for permanent grass fields, most of which are older than five years and the remainder is under annual crops of which 5,000 ha are currently used for barley. Grassland or cropland constitutes about 3.0% of total land area in Norway, and in 2012 total agricultural land amounted to 990,000 ha of which cultivated leys constituted 470,000 ha or 73% of the total grassland area. Grass leys in rotation with other crops are classified as cropland so the grassland area is actually higher than shown by Statistics Norway (2014) (Kynding Borgen and Hylen, 2013). In 2008, 60% of the grassland was owned and 40% rented land (STM2011-2012). In Sweden, short-term leys on arable land dominate the production of forage for winter feeding and grazing. Today they cover approximately 1.1 million ha, or 45% of the arable land area (Statistics Sweden, 2013). Cultivation of forage maize occurs on approximately 20,000 ha and is increasing. In Finland cultivated grasslands cover 650,000 ha or 28% of the utilizable agricultural area. Cultivated grasslands are around 330,000 ha or 13% of the utilizable agricultural area in Denmark and include grass and clover in grass-arable rotation. Other main forage crops are maize and cereal and pea whole-crop, which constitute 184,000 and 54,000 ha, respectively (Statistics Denmark, 2012).

Management of cultivated grasslands and their production potential

The livestock

The number of farm animals varies considerably across countries both in absolute terms and relative to population size (Table 3).

Table 3. Number of farm animals (total no. in thousands and per thousand inhabitants) and main agricultural produce from grassland in the Nordic countries in 2012.

Country	Dairy cows		Sheep		Horses		Goats	
	Total ×1000	Per 1000 inhabitants	Total ×1000	Per 1000 inhabitants	Total ×1000	Per 1000 inhabitants	Total ×1000	Per 1000 inhabitants
Denmark	568	101	160	29	60	11	?	?
Finland	284	52	130	24	31	6	5	<1
Iceland	25	77	476	1480	77	240	1	3
Norway	233	46	906	144	36	7	35	7
Sweden	348	37	610	64	363	39	5	<1

Country	Milk		Lamb meat, t		Beef, t		Horse meat, t	
	Total, ML	L per inhabitant	Total	Per 1000 inhabitants	Total ×1000	Per 1000 inhabitants	Total	Per 1000 inhabitants
Denmark	4909[1]	877	2000	<1	142	25	1000	<1
Finland	2188	404	950	<1	81	15	487	<1
Iceland	127	396	9920	31	4	13	1510	5
Norway	1531	305	17500	3	78	16	140	<1
Sweden	2861	300	5030	<1	121	13	1160	<1

[1] Of this 9% is organically produced

The numbers of both sheep and horses per inhabitant are highest in Iceland whereas dairy cows are of greatest importance in Denmark. In Iceland dairy cows are usually housed from early September to late May and are grazed on cultivated grass fields during summer. With the

advent of automatic milking it is though getting more common to keep milking cows inside for the whole year, with access to grazing. Most sheep farmers house their flocks from November to May. Riding horses are similarly housed from November to May but otherwise the Icelandic horse is, by tradition, kept outdoors all year around. Dairy cows are mainly fed indoors all year in Norway. The -razing period for stabled dairy cows is 8 weeks, whereas in loose-housing dairy cows should, from 2014, have the possibility to 'exercise and move around' outdoors for at least 8 weeks during the summer period. In some regions, where the main calving time is during autumn, dry cows may be grazed during summer. The housing of sheep varies much across the country depending on the duration of winter. Sheep will normally be kept indoors for one to three weeks after lambing (mid-April–mid-May), followed by some weeks of on-farm grazing before being taken to the areas of summer grazing. The sheep return to the farm in early/mid-September. The Old Norse sheep breed (*Ovis aries*) (3% of total sheep number) is being grazed all-year round, however, with strict regulations for animal welfare to secure feeding possibilities during harsh winters. Dairy cows are the main consumers of forage in Sweden and Finland. Grazing is compulsory by law for dairy cows and provides forage from May/June until the end of August/September, depending on latitude. However, supplementary feeding of dairy cows during summer is very common. In Denmark conventional dairy cows (+heifers and calves) are mostly kept in the stable the whole year, whereas the organic dairy cows are grazed at least 150 days for a minimum of 6 hours a day, in accordance with the rules.

Harvest management

Most of the fodder produced for winter feeding is ensiled either with or without additives in round bales or bunker silos in all countries. Dairy farmers in Iceland aim to take the first cut around the heading of timothy, which may occur as early as the middle of June in the more favourable regions. A second cut is usually taken on these fields around the middle of August. On sheep farms the first harvest is commonly delayed until early July and the fields are used for grazing in early spring and again in autumn for fattening the lambs prior to slaughter, rather than taking a second cut. Where two cuts are taken from hay fields about two-thirds of the fertilizer is applied in spring and one-third after first cut. Otherwise all fertilizer is applied in spring. Nitrogen inputs vary around $100 - 140$ kg ha^{-1}. In addition most farmers use animal manure to its fullest potential. In Norway fields are cut three times in the more favourable regions, in early June, late July and mid-September, whereas only two cuts are taken in the middle of June and again in the middle of August where the growing season is shorter. In Sweden and Finland winter feed is produced on intensively managed short-term leys with inputs of $150 - 250$ kg N ha^{-1} and two to four cuts per season, depending on latitude. The aim is to cut the first harvest at the beginning of heading, or at >80% IVOMD (*in vitro* organic matter digestibility). In addition most farmers use animal manure to its fullest potential. This is also the case in Denmark. There dairy farmers aim to take the first cut at a certain IVOMD or NEL (net energy lactation) concentration, and they decide the cutting time from a herbage sample and an on-line prognosis which calculates the decrease in feeding value due to growth and the effect of weather on the decrease rate. The targeted nutritive value depends on the feeding ration on the farm, but is typically 80% IVOMD. The recommended cuts are five and four for red and white clover mixtures respectively. For beef cattle, sheep and goats there is less focus on nutritive value. The maximum N-application in intensive managed grasslands is around 240 and 360kg ha^{-1} in grass-clover and pure grass, respectively. In permanent grasslands, N varies from $0 - 130$ kg ha^{-1}, depending of production level, use and nature value. Most of the applied N comes from slurry, all of which must be injected. The only other possibility is acidification and using trailing hoses.

Production capacity

According to the Icelandic Agricultural Statistics (Statistics Iceland, 2013) the mean fodder yield obtained in 2011 was 4,012 and 2,720 FEm ha^{-1} for dairy and sheep farms, respectively (Statistics Iceland, 2013). The total fodder produced on-farm in the whole country was worth a total of around 54 million Euros in 2011, which was around 55% of the total fodder costs for the whole agricultural sector that year (Statistics Iceland, 2013). Most of this was roughage obtained from hayfields. Barley produced locally has been sufficient to supply around 40% of the concentrate use in the dairy sector.

In Norway the mean fodder yield is estimated to be around 4,000 and 3,500 FEm ha^{-1} on dairy farms and sheep farms, respectively (Bakken and Johansen, 2014; A.K. Bakken, personal communication). There is no exact on-farm dry matter yield (DMY) registration, but estimated DMY and the amount of milk and meat being produced indicates a reduction in grass yield over the last few years. Important reasons are poor soil cultivation, need for drainage and use of heavier agricultural machinery. Increased conservation in round bales also may have caused increased loss due to wilting (Lunnan, 2012). Organic grassland (ley and grazed area) constituted <10 % of total area in 2012 (DEBIO, 2012). Imported feed constitutes 32% of the total feed and especially the protein fraction is increasing (SLF). If imported soya used for feed is included the imported feed is 38%. A higher degree of food self-sufficiency in Norway will require considerable changes of the diet from animal-based food to fish and plants grown in Norway (Bakken and Johansen, 2014).

In Sweden the intensively managed short-term leys yield 7 – 12 tons dry matter ha^{-1}, with a digestibility of >80% and a crude protein of around 15%. In Finland the yield is 5 – 10 tons dry matter ha^{-1}. This fodder is primarily used for dairy and beef cattle. Forage for horses and suckler cows is less intensively cultivated, and is generally harvested at a later phenological stage.

In Denmark the potential production is around 12,000 FE (90,000 MJ net energy), but due to different losses in the feed processing the net energy yield (energy consumed by the cows) was 8,410 and 2,560 FEm ha^{-1} on intensive grasslands (in crop rotation) and permanent grasslands, respectively (Statistics Denmark, 2012). For the whole country, home-grown fodder accounted for 79% and imported for 21% of the total requirements. For forage crops 97% was produced locally and 3% was imported.

The main agricultural produce obtained from this fodder in the different countries can be seen in Table 3.

Procurement of suitable plant material

Breeding for local adaptation

In a European context the main limiting factors for forage crops in the Nordic environment are a short and cool growing season and various winter stresses such as frost, ice encasement, low temperature fungi, prolonged snow cover, water-logging and low light intensity (Figure 2). Temperature and photoperiod are probably the two most important factors that control phenological development and play a decisive role for the adaptation of perennial fodder crops. This is of special importance for growth cessation of perennial crops in the autumn and obviously complicates the transfer of forage crops within the region. The photoperiod is directly related to latitude and can thus vary within the Nordic region from 14 to 24 hours and from 9 to 12 hours at the beginning and the end of the growing season, respectively. Temperature, on the other hand, can vary considerably at the same latitude, as discussed earlier, making transfer of material even more complicated. One way of dealing with such genotype-environment interactions is to calculate agroclimatic indices. These are commonly based on Accumulated Day Degrees, which are related to plant growth and development, and have been

applied successfully, together with indicies reflecting the risk of winter damage, for assessing overwintering of perennial crops in Eastern-Canada (Bélanger *et al*., 2002) and Norway (Thorsen and Höglind, 2010).

When dealing with the whole Nordic region it is sensible to define agroclimatic areas that differ in climatic conditions for plant production (SNP, 1992). One attempt has been made to construct such agroclimatic zones based on results from variety trials with timothy across the whole Nordic region (Björnsson, 1993). The outcome was five internordic zones based on similarity of results and geographic considerations. Interestingly though, in a recent study of molecular variation (SSR markers) of local Nordic timothy populations and cultivars, only 6% of the variation was between populations, and no variation was found between cultivars and local populations (Rognli *et al*., 2013a). The results for timothy are in stark contrast to meadow fescue where local Nordic populations show clear geographic structuring based on molecular variation (Fjellheim and Rognli, 2005; Fjellheim *et al*., 2009). Similarly, Finne *et al*. (2000) found highly significant genotypic variation between local populations of white clover collected from a wide range of latitudes and altitudes in Norway.

Breeding of grassland species for Denmark and different areas of Norway, Sweden and Finland has been carried out for over a 100 years. In the early days, natural selection in local material was the main method to extend the area in which a population performed well. Natural selection was likewise used to increase the resistance to various pests and diseases. Poly-crossing and progeny testing of individual plants selected for good performance followed by mass selection is nowadays the most common method. The original plant material may then be of indigenous or foreign origin, depending on the species in question. Icelandic agriculture has to a large extent depended on the import of forage cultivars. Not unexpectedly, the most suitable material has originated from the northern areas of Norway and Sweden. Northerly adapted plant material is generally characterized by low yields, slow regrowth potential after first cut and reasonable tolerance to frost and ice encasement. Timothy (*Phleum pratense*) is the only forage species for which cultivars have been bred specifically for Icelandic conditions.

Selection of appropriate species

Not surprisingly the choice of appropriate species varies depending on the geographic location. Timothy has been the major grass species in the Nordic region north of 60° N. Timothy is thus by far the most important fodder species in Iceland. It has a clear quality advantage over other grass species that are currently available for forage production in Iceland, both with respect to mean dry matter digestibility (DMD) and daily voluntary DM intake (see Helgadóttir *et al*., 2013). Similarly in Norway and Sweden and Finland, timothy is the only grass species that can be grown successfully almost anywhere because of its superior persistence (Larsen and Marum, 2006; Østrem *et al*., 2013). Where it is the main species in seed mixtures, the harvesting dates are mostly determined by the developmental stage of timothy. An increased regrowth capacity will, however, be required in a prolonged growing season. Meadow fescue (*Festuca pratensis*) is a well-adapted grass species and is frequently used in timothy-based mixtures in these countries, and in mixtures for combined cutting and grazing, smooth meadow-grass (*Poa pratensis*) is a winter-hardy species. Perennial ryegrass (*Lolium perenne*) has played a role in the more maritime regions south of 60° N. It has for many years been the most important grass species in Denmark because of high nutritive value (Søegaard *et al*., 2010) and it is also grown successfully in the southern parts of Sweden but here always in mixtures with other grasses as it is still too unreliable to be grown in monoculture. With its high biomass yield and regrowth capacity, and superior feed quality perennial ryegrass will undoubtedly become a promising option at higher latitudes with prolonged growing season and milder winters. The same is true for ×*Festulolium* hybrids (Østrem and Larsen, 2010). *Festulolium* holds a vast genetic variation (Ghesquière *et al*., 2010) and several *Festulolium* hybrids have been introduced in the last 10

years to Sweden, Denmark and Norway. Compared to perennial ryegrass it has an earlier spring growth and a lower nutritive value at the same date. Farmers therefore harvest mixtures with *Festulolium* in spring about one week earlier than mixtures with perennial ryegrass only. Targeted breeding goal for *Festulolium* is to understand the mechanisms behind the growth cessation to secure cold hardening and winter survival. For high latitudes the allotetraploid approach, including the full genome of both parental species, seems to be the most useful approach for exploiting the genetic variation, among others for winter survival, in the combination of *L. perenne* and *F. pratensis* (Østrem *et al*., 2013). The more deep-rooted tall fescue (*Festuca arundinacea*) might become a more important species also at high latitudes, due to possible drought incidences in spring/early summer in a changing climate (IPCC, 2013). When tall fescue is one of the parents, cultivars of *Festulolium* have proved a winter hardy alternative in the Nordic-Baltic region (Gutmane and Adamovics, 2008; Halling, 2012; Østrem *et al*., 2013).

Red clover (*Trifolium pratense*) and white clover (*T. repens*) are the most important legume species in the Nordic countries (Marum, 2010). In Sweden red clover dominates followed by white clover and lucerne, which is an important species in the drier eastern parts of the country. In Finland red clover is the most important forage legume. In Denmark white clover was successfully reintroduced in the 1990s after the high-N period during 1960-1990. Red clover was reintroduced a few years later. The main reason for using clover in Denmark, Finland and Sweden is the well-known higher intake of clover mixtures, demand for reduced N-application and a general perception of better quality. Today the goal for nearly all intensively managed grasslands in Denmark is to have a high content of clover, which means at least 50% in the summer period. In organic farming clover has a significant impact on the whole crop rotation system. However, white clover fatigue has been a big challenge due to a high proportion of clover in the grazing area for the cows close to the stables (Søegaard and Møller, 2005).

The importance of forage mixtures

Species mixtures are the norm in all countries. Mixtures provide yield stability over time, as well as an extended period over which a good feeding quality can be maintained. A typical mixture for large areas where grassland farming is important would contain timothy, meadow fescue, perennial ryegrass, red and white clover in different proportions depending on location, soil condition, planned sward duration and the use of the herbage. In Sweden all-grass mixtures (timothy, meadow fescue and perennial ryegrass) are grown for the production of forage for horses. Recent pan-European experiments have shown that grass-legume mixtures are more productive and show greater yield stability over time than their individual components in monoculture irrespective of fertilizer treatment (Finn *et al*., 2013) and in the northern regions higher yields were not at the cost of poorer nutritive value (Sturludóttir *et al*., 2014).

Future challenges

Climate change

Over the next 100 years the global temperature is expected to increase in the range 0.3–4.8 °C in the world, depending on climatic models, with the Arctic region warming more rapidly than the global mean (IPCC, 2013). In the Nordic countries this temperature increase is likely to be larger in northern areas than in the south, and most probably temperature will increase more during winter than summer with more of the precipitation falling as rain (Björnsson *et al*., 2008; Hanssen-Bauer *et al*., 2009). This will have various implications for adapting forage cultivars to the changing conditions both through breeding and different management schemes.

The expected changes in climate at northern latitudes will result in a longer growing season, because of earlier spring and later autumn, and higher temperatures during the growing season, both of which may lead to increased biomass production potential. However, new types of

stresses may offset the potential gain, such as insufficient hardening conditions during autumn to prepare the plants for altered winter conditions. Higher temperature in the autumn at the same light intensity will mean that the forage species are cold hardened at later stages during autumn, calling for new breeding strategies. Also during the winter deacclimation and reacclimation are important challenges due to experienced and expected temperature increase during winter (January-April) which will pose stress on plants if warm spells occur during mid-winter. In adapting forage plants to new conditions in the north, new variation in exotic material has to be looked for and the most promising material subsequently introgressed into present cultivars. As grassland agriculture in the region is a low value enterprise it would be desirable to breed material with as wide adaptation as possible so material could be used across larger areas within specific agroclimatic zones rather than focus on narrow adaptation to certain geographic regions. Such an approach would require considerable pre-breeding efforts in line with the already initiated Nordic Public Private Partnership for pre-breeding in perennial ryegrass (Rognli *et al*., 2013b).

Climate change may also increase disease pressure in forage plants. This has already occurred for leaf spots in meadow fescue, whereas e.g. crown rust is expected to become a problem on perennial ryegrass. Breeding material of vulnerable species should therefore be tested under more southern growing conditions.

Improved land- and nutrient-use efficiency

The Nordic countries must rise to the pressing challenge of sustainable intensification of their agricultural production (The Royal Society, 2009). For grassland-based agriculture this may involve improving the quality of the herbage either through breeding or optimizing D-values through more precise management strategies, thus obtaining more feeding units per land unit, as higher temperatures during summer will, without changing the management, reduce quality because of increased lignification of cell walls and lower digestibility of organic matter (Buxton, 1996). One approach would also be to increase the use of perennial ryegrass as discussed earlier and benefit from its superior feed quality and productivity. It is also important to reduce the reliance on artificial fertilizers. An obvious way is to make better use of N-fixing species and it is therefore especially important to improve their adaptation in the more marginal regions. Forage mixtures have also been shown to enhance yields compared to their individual components in pure stand (Finn *et al*., 2013) but the mixtures have to be carefully designed in order to realize their full potential in order to improve resource complementarity and increase yield.

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European grasslands overview: temperate region

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Abstract

The temperate region, from Ireland to Poland through France, Benelux and Germany is characterized by an important contribution of permanent and temporary grasslands to the Utilized Agricultural Area, with a strong decreasing gradient from West to East of the region, where they provide a range of environmental services, especially in terms of preservation of plant biodiversity. There are contrasting acreages of grasslands under organic farming. The acreage of permanent grasslands has decreased, on average, over the last decades. The acreage of pure forage legume swards has also decreased in relation to the changes in annual cropping systems. The potential of biomass production from grasslands shows a strong West-to-East pattern. The whole forage and grassland systems are relevant to the changes in potential of animal performance, especially visible in dairy cows, that is showing a steady increase in genetic potential, a concentration around a dominating breed, and strong difference among countries in terms of monthly milk deliveries. There is a range of animal PDO products that ensure an economic valorisation of grassland-based production systems. Countries of the temperate regions also developed a very important plant breeding activity dedicated to grass and legume forage species. The present paper documents all these aspects for the various countries.

Introduction

Grasslands are a major component of the landscapes in temperate regions of Europe, as they are related to a very important economic activity of animal production. In the present paper, the temperate regions include oceanic, sub-oceanic and sub-continental climatic zones. We will analyse Ireland, the UK, France, the Benelux, Germany, Czech Republic, Slovakia and Poland. Because of the large variation in climate and soil conditions, and in history, both permanent and temporary grasslands are used, the latter including a varying proportion of forage legumes. In the temperate regions there is a very limited proportion of common lands, which contribute little to feed production, even though they can play a key role for the preservation of the environment in some areas.

In this paper, we focus on the present acreage of permanent and temporary grasslands, the variation over the last decades and their production. We also describe the animals that are using this feed resource and document their number and their productivity and investigate how this influences the grassland acreage and its management. Temporary grasslands depend upon the seed sector and the potential genetic improvement of the varieties available for farmers. Alongside these supporting and provisioning services, we assess how the grasslands contribute to environmental services in the temperate regions.

The data presented in this paper were collected during the Multisward project (www.multisward.eu) (Huyghe *et al*., 2014).

1. Grassland acreage and production in temperate European regions and changes over recent years

Grasslands acreage and changes over decades

Permanent and temporary grasslands contribute contrasting proportions of the utilized agricultural area (UAA) in the various countries of the temperate region, as defined in the present study (Figure 1). Overall, permanent grasslands account for 75% to 20% of the UAA, in Ireland and Poland respectively. The proportion of temporary grasslands is lower, as it ranges from 15% in Ireland to 2% in Poland. On average, this means that permanent and temporary grasslands are a key component of the agricultural landscape in the temperate region. There is a very strong gradient from the most oceanic countries where grasslands, and especially permanent grasslands, are very abundant, to the most continental countries. This is related to the potential of biomass production (see section below).
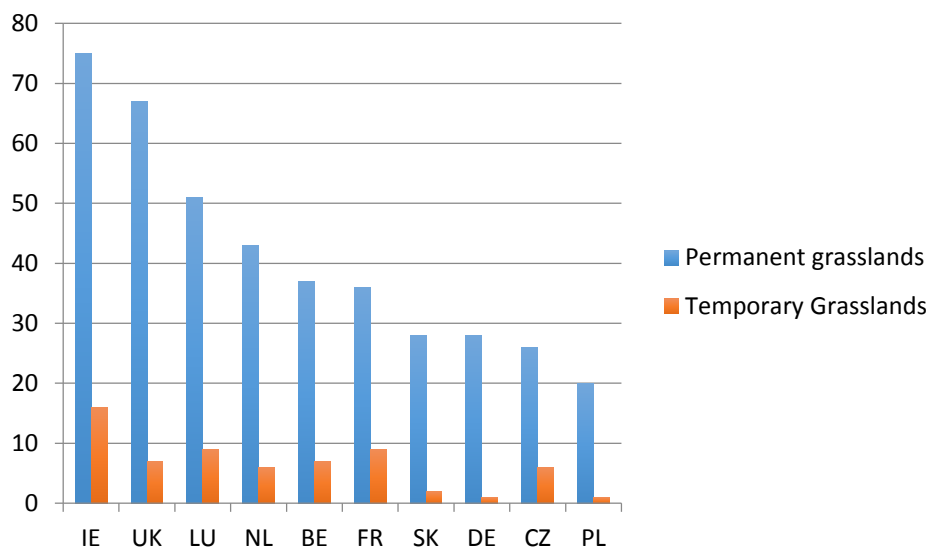
Figure 1. Share of permanent and temporary grasslands in 2009, expressed as a percentage of Utilized Agricultural Area in the countries of the temperate region considered. (Source: Eurostat, 2009).

Organic farming is rapidly expanding in Europe and herbivore production based upon grasslands, both permanent and temporary, is the main production in organic farming. Indeed, this production is easier without mineral fertilizer and pesticides and makes it possible to combine with, and provide fertility to, annual crops. However, there are strong differences among countries, both expressed as a percentage of the grasslands and in absolute values (Figure 2). Acreage of permanent grasslands under organic management is large in Germany, UK, Czech Republic and France, while most organic forage crops (temporary grasslands and annual forage crops) are the most abundant in France, Poland and Germany.

Since the 1970s, the proportion of permanent grasslands in the utilized agricultural area has slightly declined in France, Germany and Netherlands. But it has remained very stable in Poland and UK, and even increased slightly in Ireland (Figure 3). This is due to the role they play in the economic activity as a cheap feed source for herbivore production and to their ability to valorise poor soils or fragile environments. In countries where it has declined, however, the losses of permanent grasslands, expressed in hectares may reach large areas. For instance in France, the limited variation in %UAA, is in reality 3 Mha of grasslands lost between 1970 and 2007. Half of this loss is from abandonment and the other part is because of conversion to ploughed land and also as a result of urbanization (Huyghe *et al.*, 2005).
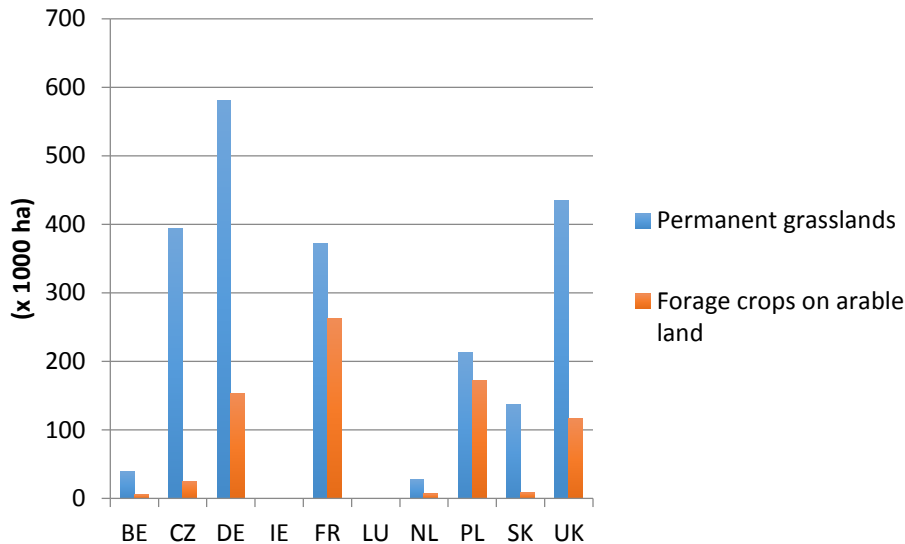
Figure 2. Organic farming area under permanent pastures and green fodder in 2011 in the countries of the temperate region considered. (Source: Eurostat, 2011).

The stability reported for the permanent grasslands is also true for temporary grasslands in all countries. The only exception is the acreage of lucerne, when grown as pure stands, which severely declined since it was a significant crops in early 1970s.
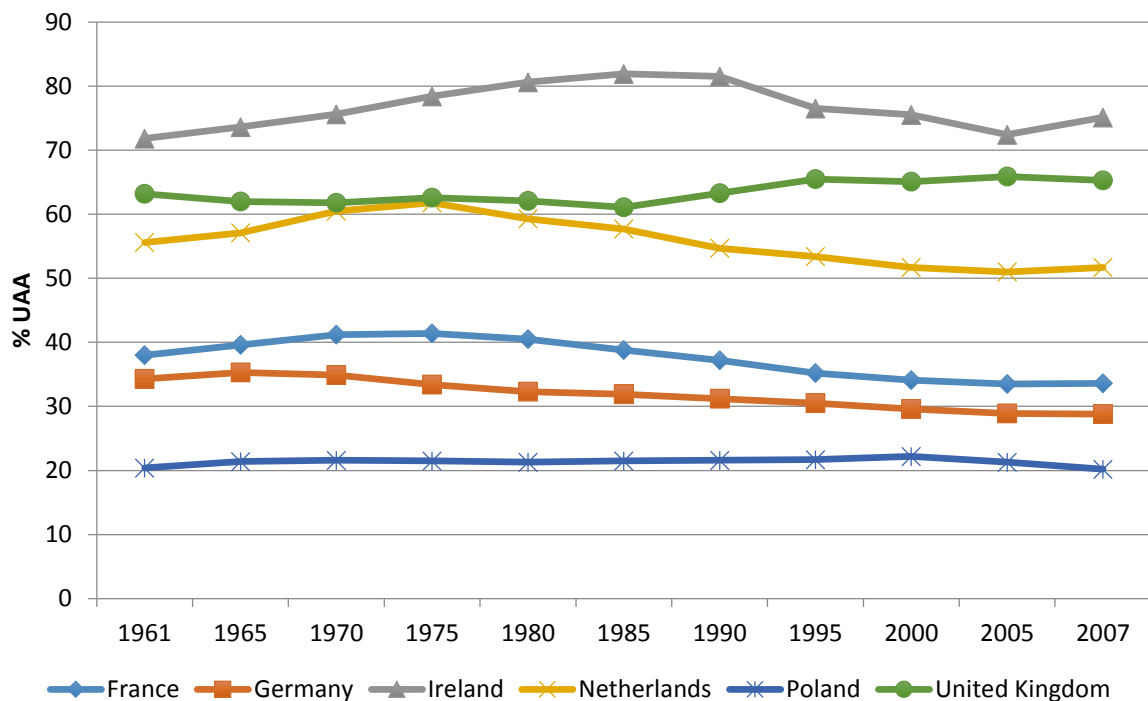
Figure 3. Changes in the proportion of the permanent grassland area in the UAA (%) in some countries of the considered temperate region between 1961 and 2007. (Source: FAOSTAT and authors' own calculations).

Even if the proportion of permanent and temporary grasslands appears stable at a country level, this may mask significant changes at the local level. This is especially important for temporary grasslands, as they are linked both to the changes in animal production and the changes in the other crops of the rotations. One of the most striking examples is the spatial distribution of lucerne in the Seine Basin in France (Mignolet *et al.*, 2012) (Figure 4). In this region, in 1970, lucerne was used both in mixed farms for feeding herbivores and in specialized cereals farms

where lucerne was used for dehydration. In 2010, the mixed farming mainly disappeared in the Seine Basin and lucerne is now only located where the dehydration factories are located. This clearly means that the contribution of lucerne, a perennial legume, is restricted to the sustainability of the cropping systems dominated by cereals. Moreover, this was accompanied by a strong reduction of animal production and a quick simplification of the rotations, down to 2 or 3 crops. Another example is the transfer from grassland to green maize in Brittany, Belgium, The Netherlands and Germany. In Belgium the grassland area decreased by 215 000 ha in the period 1970-2010, while the forage maize area increased from 18 000 ha to 176 000 ha in the same period (Anonymous, 2014).
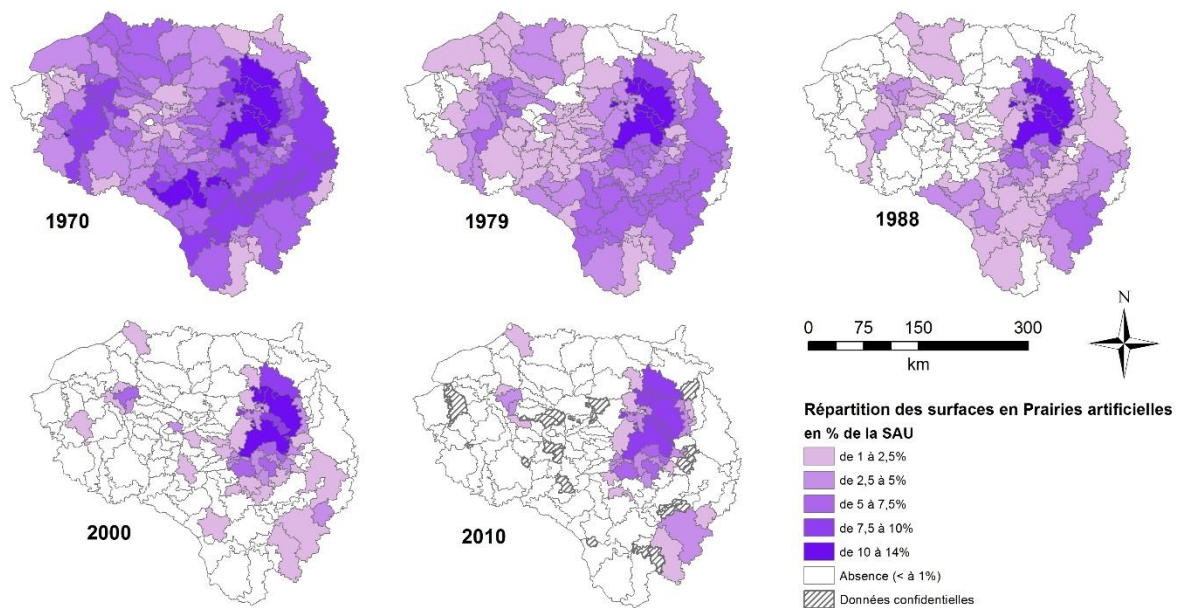
Figure 4. Lucerne production in the Seine Basin from 1970 to 2010. (Source: Schott *et al*., 2009; Catherine Mignolet, pers. comm.).

Biomass production and utilization of feed

The potential of aerial biomass from grasslands predominated by grasses was calculated for Europe, under the hypothesis of a good sward structure, a good nitrogen fertilization and a good water supply. These calculations were performed by Alain Peeters (Figure 5). The results differ slightly from the map drawn by Smit *et al*. (2008) by taking into account the soil quality. The striking feature is that the temperate region is the region with the highest potential for biomass production from grasslands, with, on average, good soil quality and adequate climate conditions. There is a slight West-to-East gradient, with higher potentials in the West due to the potential for longer growing seasons because of the oceanic climate.

This can also be related to the range of species that may be used for sowing temporary grasslands or being the most productive in the permanent grasslands. Indeed, in the Western part of the temperate region, perennial ryegrass is the predominant grass species, while in the eastern part of the considered zone, meadow fescue, smooth-stalked meadow grass and timothy will be used, and cocksfoot and tall fescue are used in France in areas that have water stress in summer. Similarly, different legume species are used in mixtures with grasses. White clover,

suitable for grazing, is the most abundant in the Western countries, while red clover, not generally suitable for grazing, is often used in mixtures in the Eastern countries of the temperate region.

A first consequence of this pattern is the mean livestock density (Figure 6). It is highest in the Benelux countries and lowest in Poland, Czech Republic and Slovakia. The lower values in these countries may also be due to the lowest levels of fertilisation on some farms, and its relevance to the lowest levels of soil fertility.

The direct consequence of this pattern may be found in the management of grasslands and especially their use by the animals. Where the production potential is high, with a long growing season, then grazing is the favourite practice as it reduces the need to make stocks of conserved feeds and, as a consequence, production costs are reduced. In contrast, where growth is limited by cold winter or summer drought, the duration of the growing season is shorter and conserved feed (silage, hay, haylage) is produced and fed either in barns or as a supplementary feed directly in the paddocks, especially to suckler cows.
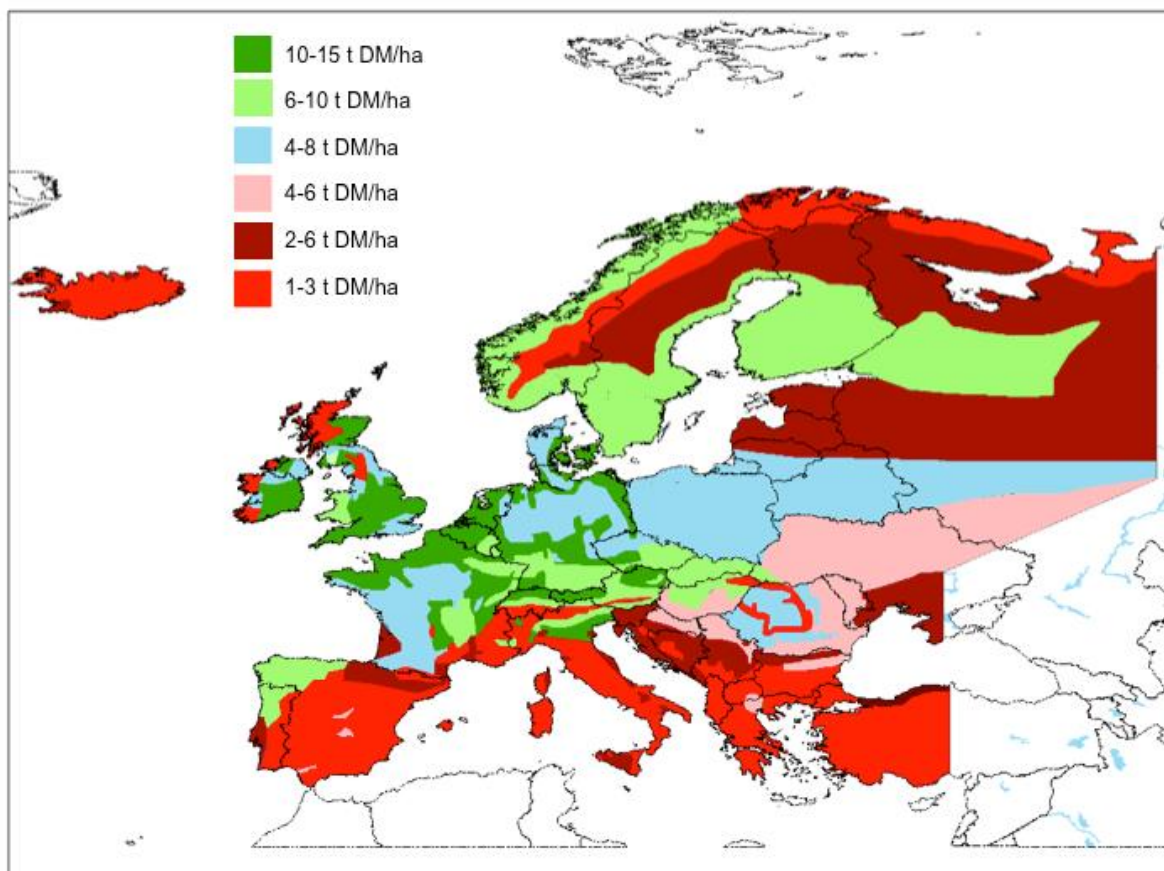
Figure 5. Production potential (annual yields in t DM/ha) of mown and heavily fertilized grasslands. (Source : A. Peeters, own calculations).
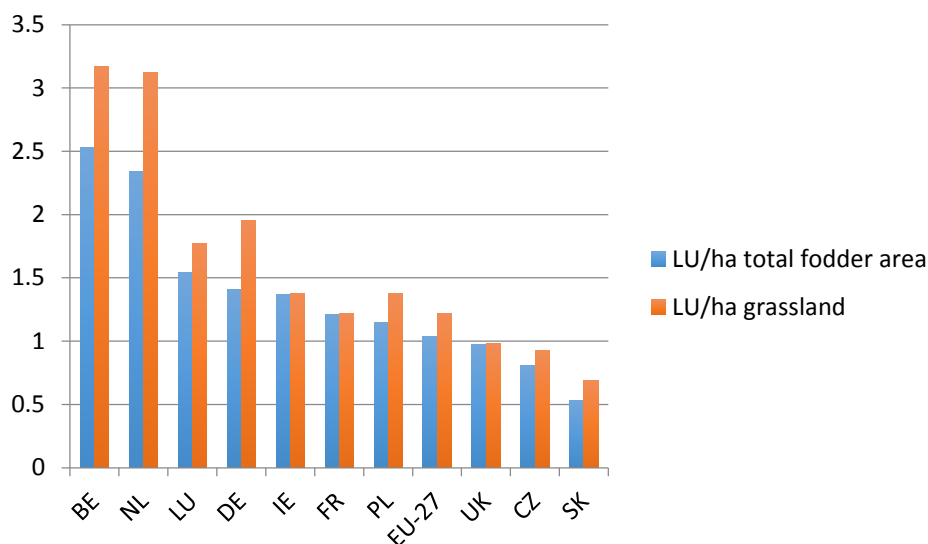
Figure 6. Grazing livestock density in the countries of the temperate region in 2007. (Source: Eurostat and authors' own calculations).

2. Animal production

The first ecosystem service provided by grasslands is the provisioning service, as grasslands ensure the production of feed for ruminants. This diet, rich in cellulose and protein, may cover a large part, or all of the nutrient requirements of cattle and sheep. In temperate regions, cattle represent the major part of the livestock units, although there are also large numbers of sheep in Ireland and especially in the UK, where they contribute 10 and 30% of the total grazing livestock respectively.

The numbers of dairy cows and beef-suckler cows are presented in Figure 7. With more than 3.5 million dairy cows, Germany and France have the largest numbers of dairy cows in Europe. The numbers of dairy cows in most countries have declined steadily since the establishment of milk quotas in 1984, and as a consequence of the high increase in milk yield per cow (Figure 9). The peculiarity of the temperate region is the high number of suckler cows (named as 'other cows' according to Eurostat). The number of suckler cows equals the number of dairy cows in Belgium, Ireland and UK and even exceeds it in France. All these countries have specialized meat breeds: Belgian Blue, Aberdeen Angus, Hereford, Limousin, Charolais, Blonde d'Aquitaine *etc*. The predominant dairy breed is the Holstein Friesian, while other dairy breeds as Normande, Montbeliarde and Braunvieh are also quite popular and have experienced increasing genetic merits that are close to those met in Holstein Friesian.

As a consequence of the growing season of grasslands and the management of the herds, the monthly deliveries of milk vary greatly among countries. Figure 8 illustrates the most contrasting patterns among the temperate region. For Ireland, there is a strong peak production in spring and summer months, where the animals graze abundant feed and this is achieved thanks to spring calving. During the winter, because of the low feed resource available for grazing, the cows are dry. The milk industry has been adapted to this large peak-to-trough ratio. In contrast, France has a fairly flat distribution of monthly milk delivery. This has been achieved over the years thanks to a differential milk price between spring and winter, encouraging the farmers to produce milk in autumn and winter. The feed resource is then ensured by provision of conserved feed stocks, mainly silage, from either grass or maize. Maize silage has to be supplemented with soy meal. Indeed, when grazing grass swards, or mixtures of grass and legumes, the animals cover their needs in energy and protein. However, this may

not be the case for the very high yielding dairy cows, for whom the energy-protein balance in grazed grass may not be optimal.
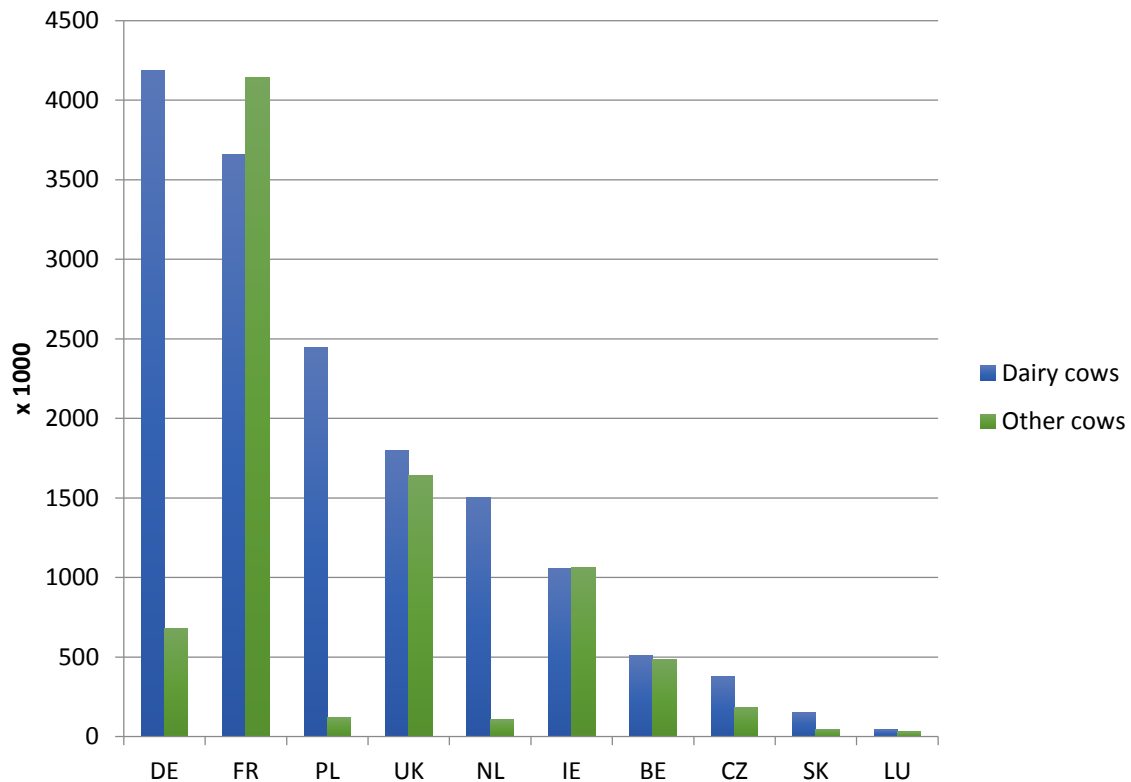
Figure 7. Number of dairy and other cows in the countries of the temperate region in 2011. Source: Eurostat, 2012.
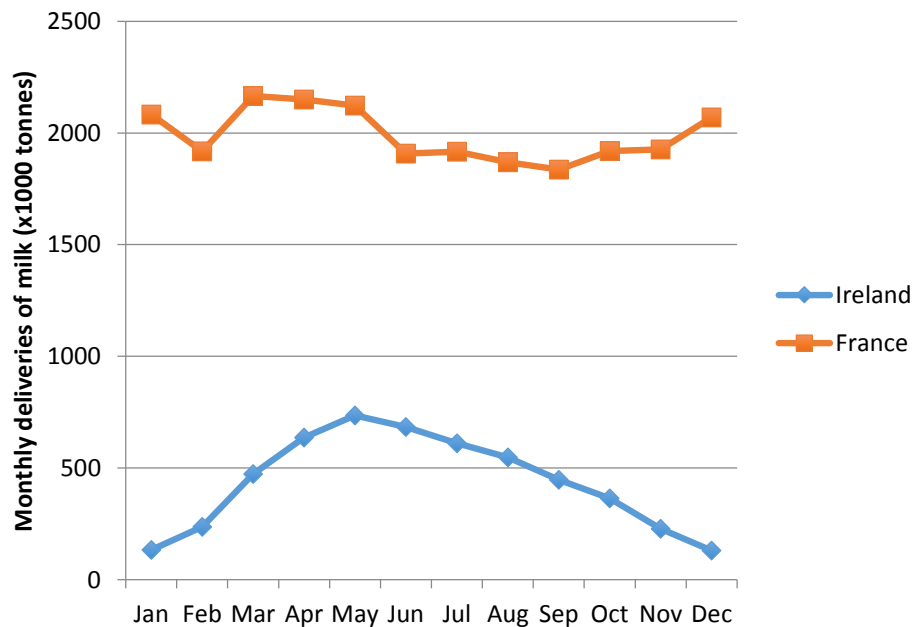
Figure 8. Changes in the monthly milk delivery of cow milk to the dairy industry in France and Ireland in 2011 (in 1000 tonnes).

When fed with maize silage whose protein content is very low, supplementation with soy meal is compulsory. As a consequence, the agronomic This underlines the coherence between the grassland acreage and management, the extra feed and concentrate resources, the management

of dairy cows and the whole dairy industry sector. It also questions the genetic value of cows and their ability to valorise grasslands. In the course of Multisward project, the potential of hybrid breed for milk-solids production, performance for grazing and reproductive performance (intervals between calvings) was underlined. And it is an extra component of this coherent system for the optimized use of feed biomass produced by grasslands.

However, the present trend in animal performance, especially milk production per cow, shows a constant increase in mean milk yield per cow and per year (Figure 9). The trend is very similar among countries of the temperate region, except Ireland where it is less steady. As a consequence, this increasing animal performance, a large part of which is due to increasing genetic merit, will not be fully relevant with a large share of the grasslands in the animal feed. A combination of grass and maize silage in a ratio, on DM basis varying between 60-40 to 40-60, and complemented with concentrates, can fulfil the requirements of energy and protein for cows with a very high milk production.

The trend of increasing performance is less pronounced in beef cattle, even though a regular increase in animal weight at slaughter is recorded. Beef cattle, in general heifers and suckler cows in particular, in all the countries of the temperate region, are mainly fed through grazing, with hay or grass silage during the winter in all the countries of the temperate region. They well valorise the permanent grasslands in many regions. In most intensive production systems, fattening bulls are kept in stables, and fed with maize silage and concentrates during the finishing period.
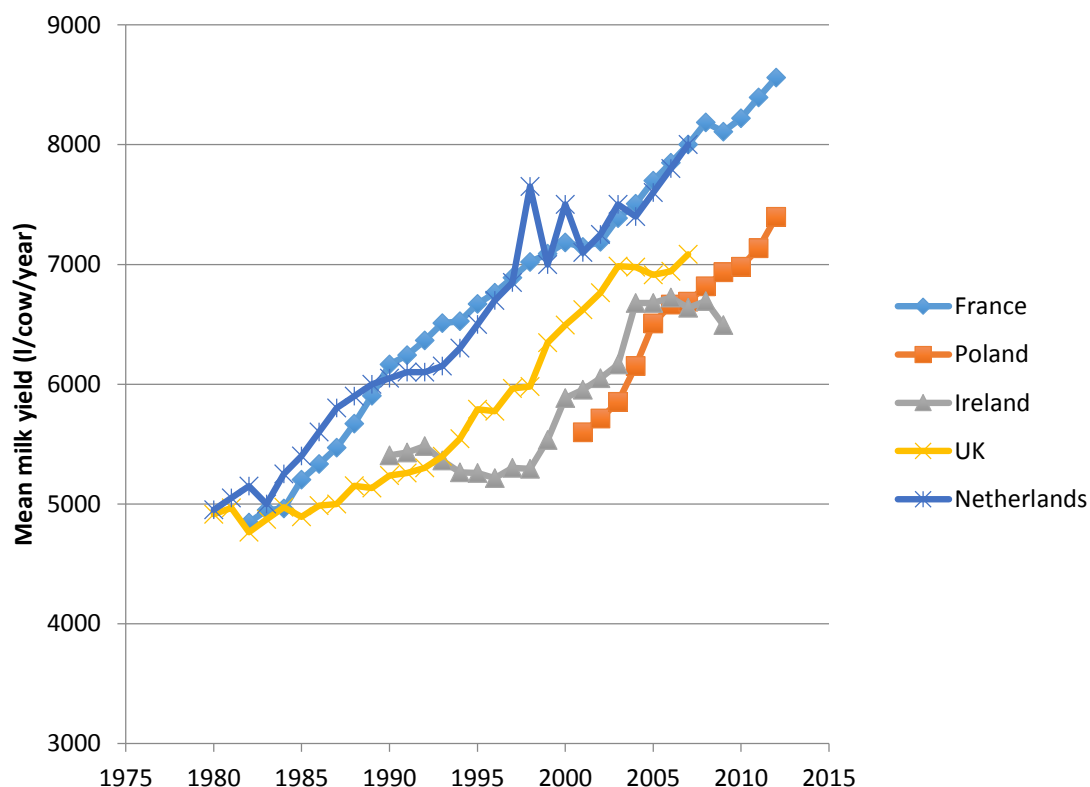
Figure 9. Mean milk yield per dairy cow in France, UK, Netherlands, Ireland and Poland over the last decades. (Source: National statistics, ICAR database).

Grassland-based dairy products have a very high value in most countries of the temperate regions. Many PDO cheeses are produced and give an additional market value. We could for instance name Comté or Beaufort in France produced in the Jura and Alps and fully based upon grazing or hay. It is also the case for Oscypek (PDO). This hard sheep's milk cheese is produced

mainly in the Podhale and Tatra regions, and was once served as payment between farmers and senior shepherds.

3. Breeding and seed production

The temperate region has a climate that is very favourable for the important breeding activity of forage species and also for seed production.

Most large forage-breeding companies are based in this region, except DLF which is located in Denmark but it also has many trial sites in the temperate region. This situation occurs because the temperate region is a major European market and varieties must be well adapted, in terms of their phenology, potential for forage production, and resistance to foliar diseases. One of the most important tasks facing breeding of fodder grasses in this region was to develop genotypes from the *Lolium* genus for increased tolerance to drought and frost by way of introducing resistance genes using species from the *Festuca* genus by interspecific crossing (Zwierzykowski *et al.*, 2004; Goliński *et al.*, 2005). In conjunction with this situation, many public research institutes are also based in the temperate region (Teagasc, IBERS Aberystwyth, INRA Lusignan, University of Hohenheim, Wageningen University, University of Poznan, for instance).

Seed production is also very important, both for forage grasses and legumes (Figure 10). Denmark is the only country that produces more grass seeds than any country of the temperate region, with 101,300 t on average between 2007 and 2009. Production is mainly devoted to grasses, with the exception of France and Czech Republic where there is also significant production of forage legumes, mainly lucerne and red clover.
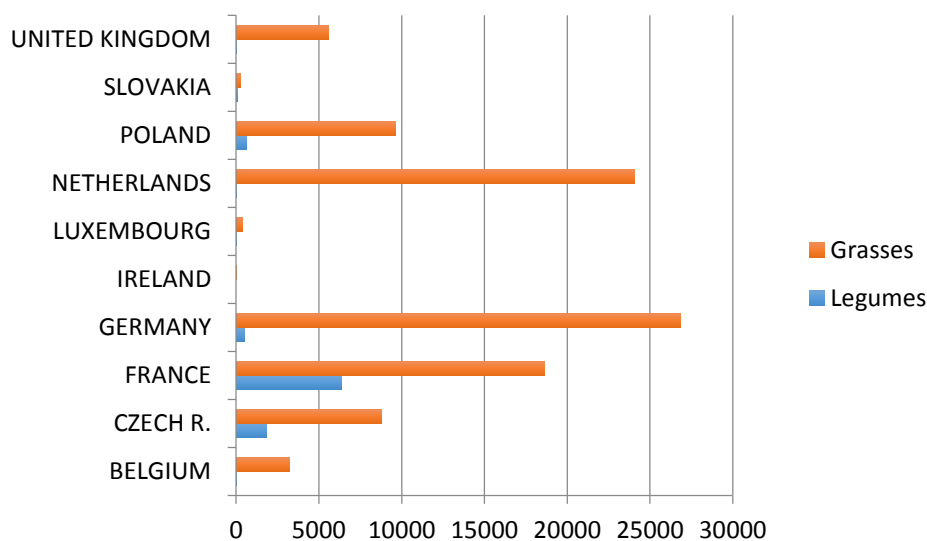
Figure 10. Mean annual seed production in the various countries of the temperate region between 2007 and 2009 (in t). (Source: National Certification Agencies).

4. Environmental services of grasslands in the temperate regions

In comparison with other European regions, the temperate zone has a fairly low number of Natura 2000 sites, but it hosts many areas of High Nature Value farmland, especially in Scotland and Wales, Massif Central and the Alps, South of Germany, and the eastern and southern parts of Poland.

These regions are those where the grassland management is less intensive and amounts of biomass production are low. In the other grasslands, the high management intensity, both in terms of soil fertility or animal management, tends to lead to a fairly low level of species diversity. Indeed, the botanical composition of pasture vegetation is strongly influenced by

management (Štýbnarová *et al.*, 2009). In the case of the remaining semi-natural pastures, botanical composition is the result of traditional agricultural activities such as haymaking or herding (Isselstein *et al.*, 2005; Maurer *et al.*, 2006; Peratoner *et al.*, 2009). Plant species-richness declines as applications of fertilizer increase, especially nitrogen (e.g., Zechmeister *et al.*, 2003) and phosphorus (Poozesh *et al.*, 2008), even if, in some situations such as ultramafic soils of Tuscany, nutrient addition tended to increase the diversity (Ricotta *et al.*, 2005). Nitrogen fertilizer levels — even when far below those applied on intensively managed grasslands — cause significant losses in sward plant diversity, with half of the number of plant species eliminated in response to fertilizations of between 20 and 50 kg N/ha/yr (Plantureux *et al.*, 2005). P enrichment presented a greater threat to biodiversity than N enrichment in research on 132 semi-natural grasslands located along a gradient of nutrient availability and atmospheric N deposition. However, as N- and P-driven species-loss appeared to be independent, the results of this research suggest that simultaneously reducing N and P inputs is a prerequisite for maintaining maximum plant diversity (Ceulemans *et al.*, 2013).

The abundance of forage legumes in the swards will have a similar effect. The impact of grazing management on grassland biodiversity has been documented in many studies (e.g., Rook and Tallowin, 2005). Nutrient depletion can be accelerated by haymaking. However, atmospheric deposition of nutrients is increasing and such deposits are believed to slow down the effects that extensification has on biodiversity (Plantureux *et al.*, 2005).

Today, in regions with intensified agriculture such as the countries of the temperate region, semi-natural grasslands persist only on a low proportion of the total grassland area. Their preservation is a primary goal for nature conservation (Isselstein *et al.*, 2005), such as through the Habitats Directive (1992) or the international treaty drawn up at the Ramsar Convention on Wetlands (1971). Semi-natural grasslands have persisted mainly on locations that are less suitable for agriculture because of biotic and abiotic constraints (Pärtel *et al.*, 2005). Yet, areas of semi-natural grasslands still exist in Poland (Veen *et al.*, 2001; Goliński and Golińska, 2011) where they must be preserved and possibly valorised economically, for instance through recreation of special and well-identified animal products (see above).

Erosion is becoming a major issue for European soils (Souchère *et al.*, 2003), as an effect of the change in land use. Grasslands are a key asset for limiting soil erosion because of their ability to reduce run-off, thanks to the permanent soil cover. Within the framework of good agricultural and environmental practices, permanent grassland is strongly recommended and in some regions is obligatory and financially supported on slopes with a minimal gradient. However, because of the reduction in the area of permanent grasslands in the temperate region countries, this issue will become increasing important and should be considered as a benefit of grasslands.

Conclusions: the main drivers of the changes

Grasslands are experiencing dramatic changes in the countries of European temperate region. All the changes are occurring in a very relevant way related to an increasing animal performance and to an increasing productivity of human work in larger farms. Beyond the search for a higher biomass productivity, which is possible in this region because of the soil and climate conditions, there is also a risk of grassland abandonment or ploughing, as herbivores, especially high genetic-merit dairy cows, are increasingly fed with maize silage supplemented with soya-based concentrate.

The Common Agricultural Policy has been fully in line with this trend. Quotas, established in 1984, have provided security for dairy farmers and, in some countries, have slowed down the concentration of milk production and the dairy industry in the most favourable zones for biomass production, milk processing and export. The future disappearance of quotas will offer the possibility for a quick increase in mean production per dairy farm and, simultaneously a

reduction in the number of dairy farms, which will be concentrated in these favourable zones. These trends will, however, endanger the large regulating, provisioning and cultural ecosystem services provided by grassland and grassland-based production systems in the rest of the temperate region.

As a consequence, it is very important that the future European and national policies of the countries in the temperate region take into account both the economic and environmental services provided by grasslands and grassland-based systems, and also consider the social aspects for farmers involved in herbivore production and of the whole of society which derives a huge benefit from grasslands.

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European grasslands overview: Mediterranean region

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Abstract

The paper reviews the main traits of the grasslands in the Mediterranean basin, a global biodiversity hotspot where almost 22,500 endemic vascular plant species, more than four times the number found in all the rest of Europe, have been described. In this context the grassland-based systems are no longer seen exclusively as livestock production enterprises but as multiple-use systems with important consequences for the global environment. Plants are subjected to very low rainfall during the spring-summer period (2-6 months) resulting in a 300-1000 mm water deficit that negatively affects plant survival and crop production. For this reason the traits of species to cope with summer drought are discussed with special emphasis on drought escape in annual species and seed bank management, as well as drought survival in perennial grasses. The agronomic role and importance of legumes in the grassland mixtures are also described pointing out the grass-legume interactions. The prediction of the influence of the global warming on Mediterranean pasture grasses is analysed, as well as the contribution of grassland to environmental issues (multifunctionality). With this regard the results of the role of the grasslands on soil erosion control and carbon sequestration are reported. The conservation of biodiversity in grassland species is discussed. Finally, the use of grasslands as source of biomass for bioenergy and bio-refining are also presented.

Keywords: drought escape, summer survival, multifunctionality, soil erosion, carbon sequestration, biodiversity, bioenergy

Introduction

There are five areas of the world with Mediterranean-type climates: the Mediterranean basin itself, South Africa, California, Chile and southern Australia. By far the largest is the Mediterranean basin, it covers approximately 2.5 millions km^2; about half of the Mediterranean areas are located on the European side and over than 50% is represented by grasslands and rangelands (Eurostat, 2010). They are important ecosystems that have traditionally played an important role in the evolution of human societies (Jouven *et al*., 2010). The fact that the Mediterranean basin is a global biodiversity hotspot with an extremely high number of endemic plant species is strongly related to the long-term management practices. Livestock grazing is the major factor in these ecosystems, which in addition to the quantity and quality of forage, strongly affects vegetation dynamics, species, and landscape diversity (Perevolotsky, 2005). Animal production systems in Mediterranean Europe are dominated by small ruminants and beef cattle belonging to local races which, with respect to dairy cows, exploit better unfavourable areas and shrublands under all-year-round open-air grazing. Moreover, Mediterranean grassland-based systems are no longer seen exclusively as livestock production enterprises but as multiple-use systems with important consequences for the global environment.

Elevation greatly affects local climate and ecology in this area and vegetation can be distinguished on the basis of thermal criteria (Barbero and Quezel, 1981). Plants are subjected

to very low rainfall during the spring-summer period (2-5 months) resulting in a 300-1000 mm water deficit (Volaire *et al*., 2009) that negatively affects plant survival and crop production. The stress imposed during summer months is the most important environmental constraints for plant growth in association with large intra- and inter-annual variability of rainfall distribution. This rainfall pattern induces a highly seasonal growth of plants during spring months (February-March/May-June) and reduced growth or dormancy of plants during summer months, with a second growth period occurring in autumn.

In this paper we consider a recent classification proposed by Peeters *et al*., (2013) which defines grasslands as 'land devoted to the production of forage for harvest by grazing/browsing, cutting, or both, or used for other agricultural purposes such as renewable energy production'. The conservation of grasslands is important not only as a feed source but also because they support biodiversity, contribute to the reduction of CO_2 levels from atmosphere, as they act as a carbon sink, and generate several environmental and economic services such as prevention of fire risk, recreational activities and tourism (Carrillo *et al*., 2014). Therefore, the characterization of the environmental threats that affect European Mediterranean grasslands and the ability of species to cope with climate change is a fundamental step to increasing resilience of grassland.

Designing resilient and sustainable grasslands: traits of species to cope with summer drought

Drought escape in annual species and seed bank management

Mediterranean semi-natural grasslands are dominated by annual species well adapted to the highly variable Mediterranean climate because they produce a huge amount of seeds that can survive for a long time in the soil seed bank that plays an important role in the composition and conservation of plant communities (Koukoura, 2007). The annual composition and abundance of species is subjected to wide fluctuations, depending on the number of seeds produced and their dormancy type and degree, the satisfaction of their germination requirements, the soil physical and microbiological characteristics, the pattern of environmental factors, grazing, burning and other soil disturbance factors that can influence the total amount of seed produced by the plant community (Long *et al*., 2014). For example, increasing air temperatures may affect seed bank through an increased soil moisture evaporation, which induces a reduced seedling survival and related species richness (Ooi, 2012).

Drought escape is the main adaptive strategy that is exhibited by annual pasture or forage species surviving during the dry period as seed. These species frequently exhibit seed dormancy, which has been defined as an 'internal condition of the seed that prevents its germination under even favourable water, thermal and gaseous conditions' (Benech-Arnold *et al*., 2000). This condition of the seed to remain viable but quiescent allows the seed itself to persist in the soil for months or years until the environmental conditions permit germination (Baskin and Baskin, 2004). Temperature is the major environmental factor regulating seed dormancy and germination, and seasonal changes in temperature are responsible for the loss and cycling of dormancy (Hilhorst, 1998). In legumes, seed dormancy is determined by the presence of a water-impermeable coat. Hardseededness is believed to be controlled by both genetic and environmental factors occurring during seed development (Clua and Gimenez, 2003). In this regard, the results of a study conducted on *Medicago rugosa* Desr. and *M. orbicularis* Bartal, two annual pasture legumes quite common in the Mediterranean basin, revealed that the level of hardseededness may greatly depend on the intensity of plant water stress occurring during seed development and filling, and that more permeable and promptly germinating seeds could be produced by modifying the water balance (Norman *et al*., 2002; Patanè *et al*., 2008). The softening of hard seeds differs between and within species and in

many situations the level of hardseededness may be less important than the softening pattern, as found by Porqueddu *et al*. (1996) in *M. polymorpha* L.

Hardseededness is responsible for the differences in seed germination rate through the year in natural conditions; however, it may represent a limit when a prompt germination is required, i.e. sown pasture. Both mechanical and chemical scarification are useful for the breakdown of hardseededness, but the outcome is influenced by seed traits. In an experiment, seeds of *M. rugosa* exhibited a 65% germination after 35 min soaking in a 70% concentrated sulphuric acid solution whilst seeds of *M. orbicularis* did not germinate under the same chemical treatment (Patanè and Bradford, 1993). These results were supported by the different thickness of the palisade layer of the seed coat, as observed at SEM (Patanè and Gresta, 2006), which is more than two-fold greater in *M. orbicularis* when compared to *M. rugosa.*

In addition to meteorological factors, grassland management practices may also have huge effects on soil seed bank dynamics but their effects are controversial. Under grazing, the type of regime (continuous vs. seasonal), grazing pressure (low or heavy) and grazing season have different effects on seed bank density and on the different functional groups of plants, with heavy grazing being unfavourable to the seed bank of annuals if grazing is prolonged during the period of seed setting (Sternberg *et al*., 2003). Nonetheless, the maintenance of Mediterranean grasslands with a high degree of plant biodiversity depends on frequent grazing that controls the growth and predominance of a few dominant tall grass species (Sternberg *et al*., 2000). In general, intensive grazing seems to favour Mediterranean annual legumes, especially subclover-based pastures. Although winter grazing is advantageous to seed production, avoiding excessive predation of seed by livestock grazing stubble is also a critical aspect in legume persistence. The persistence is not only related to the seed yield but also to the pod and seed characteristics of each pasture legume, e.g. seed size, seed dispersal capacity and seed burial ability.

Drought survival in perennial species

Drought tolerance is expressed in slowly growing perennial plants (Chaves *et al*., 2003). Drought tolerance in perennial species is also of great agronomic importance. In fact, the production period of annuals is short and could be further compromised by climate change. The use of perennial forage species could extend the feeding season, increase yield during winter and also enable production in late spring in the presence of residual soil moisture (Annicchiarico *et al*., 2011; Annicchiarico *et al*., 2013; Volaire, 2008; Volaire *et al*., 2013). There are, however, very few cultivars that are suitable for Mediterranean severe drought conditions (Annicchiarico *et al*., 2013; Volaire *et al.*, 2013). The required characteristics, as reported by Volaire *et al*. (2013), are not to grow during the drought period but survive across the droughts (Annicchiarico *et al*., 2011). In Mediterranean and temperate plants a combination of plant strategies to overcome drought are present (Volaire and Lelievre, 1997; Poirier *et al*., 2012). Plant adaptations include dehydration delay (avoidance), for example by increased root development and water uptake (Volaire and Lelievre, 2001), dehydration tolerance by a superior osmotic potential through solute accumulation and osmotic adjustment (Volaire, 2008) and plant summer dormancy, an adaptive response that enables survival during the most threatening seasons because it maintains viability of meristems and prevents regrowth during cases of occasional summer rainfall (Volaire *et al*., 2009; Lelièvre *et al*., 2011).

Summer-dormant genotypes showed a superior survival after severe and repeated summer droughts (Norton *et al*., 2006; 2012). Summer dormancy is an endogenously controlled process that results in the cessation or reduction of leaf growth in summer and the senescence of mature herbage in some genotypes of perennial pasture species, such as cocksfoot (*Dactylis glomerata*), tall fescue (*Festuca arundinacea*), bulbous canary grass (*Phalaris aquatica*) and perennial ryegrass (*Lolium perenne*), and is expressed under high temperatures and long

photoperiods and, in the genotypes which have the trait, expression will occur independently of soil moisture level (Norton *et al.*, 2006).

Copani *et al.* (2012) carried out a field experiment in order to investigate the genetic diversity and the presence of summer dormancy in several wild Sicilian populations of cocksfoot. The results highlighted that a high level of summer dormancy was present in most populations; however, two populations showed an incomplete dormancy and two showed the absence of dormancy. The same authors observed a relationship between summer dormancy level and the microclimate of the collection site. Most of the summer-dormant populations came from sites with rainfall < 600 mm year^{-1} and a dry period > 120 days (Copani *et al.*, 2012).

Norton *et al.* (2012) suggested selection for summer dormancy and dehydration-tolerance genotypes prior to field screening, as this is highly likely to improve overall persistence. A method based on germination response to photoperiod to differentiate summer-dormant from summer-active types of perennial grasses has been developed by Malinowski *et al.* (2008). The authors observed that the germination response to photoperiod was associated with summer dormancy type and not necessarily with the climate where grasses developed. Moreover, Annichiarico *et al.* (2011) highlighted the need to select different adaption targets, plant types and genetic resources for the different environments. For instance, summer-dormant genotypes of *D. glomerata* subsp. *hispanica* have a prevailing interest for northern Africa. Conversely, non-dormant or incompletely dormant Mediterranean cultivars of *D. glomerata* subsp. *glomerata* are best adapted for southern Europe, especially when targeted to moderate crop duration (3–4 years). Nonetheless, completely summer-dormant germplasm could gain adaptive potential for Mediterranean-climate European regions in the future, to mitigate the effects of the predicted increase in drought due to climate change. The concurrent use of plants using different strategies to overcome drought is one of the adaptation strategies proposed by Kreyling *et al.* (2008), useful to establish permanent and multi-specific grasslands that ensure ecosystem stability, in order to enhance the sustainability of agricultural production and ecosystem services (Volaire *et al.*, 2013).

The role and use of legumes

Naturally occurring forage legumes (annuals and perennials) are well-nodulated, and their root nodules are active in fixing N_2. As a consequence, legumes contribute to increase fertility of soils, help to maintain the organic matter of soil and improve the physical conditions of soil, promoting efficient low input and low cost production systems and thereby reducing the need for inorganic fertilizers. This is true mostly for the European Mediterranean regions characterized by a favourable climate for legume growth and very poor soils (Porqueddu, 2001; Sulas, 2005).

Testa and Cosentino (2009) evaluated, using the isotope dilution method, the amount of nitrogen fixation of three legumes (*Medicago sativa*, *Trifolium subterraneum* and *Vicia faba* subsp. Minor). The percentage of nitrogen derived from the atmosphere (Ndfa) ranged from 94% to 84% (respectively in the first and second year) in *M. sativa*, from 69% to 81% in *T. subterraneum* and from 91% to 93% in *V. faba*. Other similar researches carried out by Cosentino *et al.* (2003) highlighted high values of nitrogen derived from atmosphere in sulla and alfalfa, corresponding from 136 and 162 kg ha^{-1} of nitrogen fixed by sulla (in the first and second year, respectively) and 67 and 184 kg ha^{-1} of fixed nitrogen in alfalfa. Similar values were obtained by Sulas *et al.* (2009) that in Northwest Sardinia (Sassari) and Central Western Italy, obtained 113 kg ha^{-1} and 130 kg ha^{-1} of nitrogen fixed on *Sulla coronaria* (L.) Medik (sulla). Moreover, it is well known that legumes improve nutritional quality of forage, reducing the need for concentrate feeds, also if their nutritive value may be influenced by the presence of anti-quality factors (Papanastasis and Papachristou, 2000). In addition, if present in moderate concentrations, some secondary metabolites of legumes, i.e. condensed tannins, can enhance

forage nutritive value by playing a role in promoting amino-acid absorption in the intestine, decreasing nitrogen excretion, and they can reduce GHG emission in atmosphere and the loads of gastro-intestinal parasites (Piluzza *et al*., 2014). Legume forage can influence the quality of meat in cattle (Maughan *et al*., 2014) and increase daily average liveweight gain, linolenic and linoleic acids proportion and unsaturated to saturated fatty acids in lamb meat (Fraser *et al*., 2004).

In the presence of a natural seed bank of pasture legumes in a grassland, a single or a yearly-repeated phosphorus fertilization, without any over-seeding, may be sufficient to gain satisfactory productive results (Bullitta *et al*., 1989; Henkin and Seligman, 2000) and increase the resilience of the system.

In the last forty years, the annual self-reseeding legumes selected in Australia have been also increasingly utilized in Mediterranean Europe, i.e. subterranean clovers and medics, mainly for the improvement of low quality native pastures in agro-pastoral systems, although these commercial varieties have sometimes been shown to be unfit for the different climatic conditions and management systems of southern Europe (Sulas, 2005, Porqueddu *et al*., 2010, Salis *et al*., 2012) and an effort is required to promote the selection and the seed multiplication of native genotypes. A review on annual self-reseeding legumes and their role in Mediterranean farming systems was done by Porqueddu and Gonzales (2006). Among perennial species, the well-known alfalfa (*Medicago sativa* L.) is a drought-tolerant perennial pasture legume that is valuable in many farming systems due to its ability to produce summer fodder. The deep taproot system allows alfalfa to access to deep soil moisture, tolerate long dry periods, and respond quickly to summer rainfall. These characteristics were confirmed by Testa *et al*. (2011) who evaluated the effect of harvest time and soil water content on three varieties of alfalfa in a four-year experiment. They found that alfalfa could be used for the improvement of qualitative and quantitative characteristics of forage in 'low mountain' areas of the Mediterranean, thanks to the slight decrease in yield and quality in rainfed conditions. Furthermore, this research highlighted that, by combining two harvest times, producers can ensure fresh forage from May to November. Yet, some perennial legumes are able to escape summer drought and regrow at first rain in autumn, as in the case of sulla and *Onobrychis viciifolia* Scop. (sainfoin), and their exploitation offers an opportunity to stabilize both grassland production and forage quality (Sulas, 2005; Demdoum *et al*., 2010; Re *et al*., 2014). More recently, Australian research has focused on deep-rooted and drought-tolerant perennial legumes (e.g., Caucasian clover, stoloniferous red clover, tallish clover, *etc*.), which also have a high feeding values that decline slowly with maturity. Recent research indicates that the Mediterranean *Bituminaria bituminosa* L. (syn. *Psoralea bituminosa* C.H. Stirton) has potential as a forage legume for disadvantaged areas of the Mediterranean (Martínez-Fernández *et al*., 2012). This perennial legume is drought tolerant due to a deep root system and other physiological adaptive traits (Castello *et al*., 2013). It grows and remains green all-year-round even during summer and autumn and is assumed to be tolerant of heavy grazing (Sternberg *et al*., 2006). The preliminary results obtained in different Mediterranean regions are very encouraging in view of the valorization of *Psoralea* as perennial forage legume for marginal rainfed areas (Real and Verbyla, 2010; Porqueddu *et al*., 2011). Growing *Psoralea* in permanent dense stands may provide alternative sources of highly nutritious forage (Reaside *et al*., 2013). Nonetheless, it has been estimated that approximately 70% of freshly harvested seeds of this species are hard and exhibit a three-months primary dormancy, the causes of which are still unknown (Castello *et al*., 2013).

Grassland mixtures and grass-legume interactions

Under sub-optimal and variable environmental conditions, as occur in many pastoral farming systems of the Mediterranean basin, the maintenance of high levels of inter- and intra-specific diversity is essential to achieve satisfactory and persistent pasture swards, and also for the

control of weeds and replacement of forage plants damaged by drought or biotic adversity. When improvement of the native sward is needed, the use of mixtures of pasture species rather than a single species seems more appropriate (Dear and Roggero, 2003). Nonetheless, there has been little experimentation and there is little information available on production, biomass composition and effect of grazing on the persistence and environmental impact of mixed-legume swards (Rochon *et al.*, 2004).

There are many advantages connected to the use of forage mixtures in relation to agronomic (Cosentino *et al.*, 2003), environmental and economic aspects (Malhi *et al.*, 2002). Due to the utilization of nitrogen symbiotically fixed by legumes (Whitehead, 1995), grass crops, when grown in association with legumes, may improve forage dry matter and protein yield even if the efficiency of this utilization could be strongly influenced by genotypic and environmental factors (Cassaniti *et al.*, 2003a; 2003b). Testa *et al.* (2006) studied the nitrogen transferred from a legume (alfalfa or *Lotus corniculatus*) in a binary mixture with a grass (Italian ryegrass) at different sowing rates. The estimation of % Ndfa in the grass component showed different behaviour in relation to legumes involved in the mixtures and its density. In alfalfa, the highest sowing density positively affected the amount of nitrogen derived from the atmosphere in the grass component.

A coordinated field experiment across 31 European sites confirmed that a significant yield gain was obtained when four-species mixtures were used, yielding more than the highest-yielding monoculture at most sites, including the Mediterranean ones (Finn *et al.*, 2013). Porqueddu and Maltoni (2007) found that the use of species (*L. rigidum*, *D. glomerata*, *M. polymorpha*, *M. sativa*) belonging to different functional groups (fast and low establishing grasses, fast and low establishing legumes) enabled the achievement of higher yields, a better seasonal forage distribution, and a better weed control, and higher forage quality and lower seasonal variation with respect to pure stands were assessed (Maltoni *et al.*, 2007). In the Mediterranean environment the advantage of using mixtures of grass and legume was confirmed by Riggi *et al.* (2006), who tested legume and grass mixtures at different sowing ratios. A stable pasture mixture could be obtained also with a mixture of summer-dormant and summer-active species or varieties, so that the available moisture in soil could be exploited throughout the year (Norton and Volaire, 2012). The stability and persistence of each component in the mixture is another point to be considered when determining mixture composition. Several studies have shown that in mixtures based on annual medics, *T. michelianum* and subclover, the latter species prevails from the second year onwards (Porqueddu *et al.*, 2004).

Prediction of climatic changes on Mediterranean pasture grasses

Climatic models forecast climate change will have a great impact on agricultural systems and production, with different intensity depending on the region. Warmer and drier conditions by 2050 are predicted for Europe. Southern Europe is likely to experience the largest yield losses (-25 % by 2080 under a 5.4 °C warming), with increased risks of failure especially for rain-fed summer crops (IPCC, 2013). Increased inter-annual variability may be another significant aspect of climate change, and this is of high ecological relevance. Climate change is considered likely to have a long-term great impact on all plants, primarily those whose persistence depends on the soil seed bank, e.g. grassland plants. This fact involves an assessment of biodiversity in response to future scenarios, in order to identify those species that are less susceptible to climatic changes. The adaptive capacity of these species, however, depends on several factors, including their adaptation speed to changed climatic conditions. However, a meta-analysis carried out by Dumont *et al.* (2014) did not reveal any variations in the response of grasses, forbs and legumes to elevated CO_2, warming and drought under Mediterranean conditions. Climate change is thus not expected to directly affect the chemical composition of grassland species, but could shift grassland botanical composition.

The strong relationship between climatic factors and seed dormancy and germination indicates that climatic changes will certainly have impact on seed banks, which ensure survival of the seed population, particularly under uncertain climates. Besides temperature, water is crucial for seed germination and its availability greatly affects seed dormancy and germination speed (Bradford, 2002). In a climatic change scenario it may be essential to predict seed germination under specific thermal and water environmental conditions, and modeling germination response to modified environmental conditions may contribute to increasing the accuracy of predicted climate change impacts on each plant species (Ooi, 2012). Thermal time, hydrotime and hydrothermal time models have been proposed to predict how any fraction of the seed population will respond to an environmental disturb or change, in relation to its physiological state (Bradford, 2002). To predict long-term consequences of climatic changes on species distribution and potential extinction, a deep study is required of relationships associating these changes with mechanisms regulating seed bank longevity in ecosystems where population dynamics are driven by environmental factors.

Contribution of grassland to environmental issues (multifunctionality)

Soil erosion control

Mediterranean areas are susceptible to soil erosion due to orographic and climatic conditions. Rainfall is mainly concentrated in the autumn-winter period, thereby supporting the cultivation of autumn-winter crops. According to Plan Bleu (2003) the area of land subjected to soil erosion in the Mediterranean environment covers 1,309,000 km^2, equal to 15% of the land of Mediterranean countries. The long dry period followed by heavy bursts of erosive rainfall, falling on steep slopes with fragile soils, resulting in considerable amounts of soil erosion that, especially, through the loss of organic matter and nutrients leads to a reduction of cultivable soil depth and soil fertility (Van Rompaey *et al.*, 2005). This causes a loss of productivity, which initially is replaced with increased applications of fertilizers, but at the end this can lead to land abandonment (Cerdan *et al.*, 2010). In this environment the growing cycle of winter cereals and annual forage crops (e.g. oats-vetch) determines a lack of soil covering during the first rains which occur in the early months of autumn and winter. These aspects are confirmed and highlighted by an ongoing research carried out by Cosentino *et al.* (2008, 2011) from 1996 in Sicily on a slope of 26-28%. In each year the effect of twelve cropping systems, among which some were meadows, were evaluated. The highest annual values of soil losses were observed in the annual tilled crops, 23 t ha^{-1} yr^{-1} in the average of the crop rotations legume-cereal (d. wheat)-brassica, 15.5 t ha^{-1} yr^{-1} in the crop rotation d. wheat - d. wheat - set aside, and 11 t ha^{-1} yr^{-1} in the monoculture of d. wheat. Very low soil losses were observed in the plots managed with perennial crops, alfalfa (0.15 t ha^{-1} yr^{-1}), Italian ryegrass in pure stand and in mixtures with *T. subterraneum* (1.8 t ha^{-1} yr^{-1}) and tall fescue followed by subterranean clover (1.3 t ha^{-1} yr^{-1}). The perennial *Miscanthus* and *Medicago arborea* reduced soil losses to 0.1 t ha^{-1} yr^{-1}. Introducing in this environment the use of conservative techniques (sod seeding) in the cultivation of cereals, allows some reduction in soil losses (2 t ha^{-1} yr^{-1}) maintaining the yield that is not different to that of conventional tillage. Porqueddu *et al.* (1994), in similar experiments carried out in Sardinia, showed that on a 30% slope, average annual soil losses were ten and twenty times higher in annual forage crop and bare soil respectively, compared to permanent grassland. In addition, annual forage crops did not give great advantage in terms of forage yield compared to that of fertilized permanent pasture on slopes.

Carbon sequestration

The role that natural rangelands play in the global carbon cycle is extremely important, accounting for 10–30% of the world's total soil organic carbon (Brevik, 2012). As reported by Doran and Zeiss (2000) the thin layer of soil covering the surface of the earth is a major interface between agriculture and the environment and represents the difference between survival and extinction for most land-based life. In the soil, decomposition processes, as mediated by organisms in soil, play a predominant role in completing the cycle of life, in recycling of the building block nutrients to plants, and C as CO_2 to the atmosphere (Doran, 2002). These assumptions were confirmed by a long-term field experiment carried out by Cosentino *et al.* (2013): in all the plots where perennial or forage crops were cultivated it was observed that there was a higher amount of carbon stored in the soil. Comparing the soil organic matter (SOM) content observed in two typical Mediterranean cropping systems involving durum wheat (two years of durum wheat followed by fallow) with plots managed with grasses (*Lolium multiflorum* Lam.) or forage legume (*M. sativa*), they observed a slight decrease in the SOM of the plots cultivated with durum wheat, from 1.34% to 1.18% (the average of the 0-40 cm soil layers) while a slight increase was observed in a plot managed for 7 years with *L. multiflorum* and then with *Lolium* intercropped with subterranean clover (from 1.58 to 1.66%, representing an equivalent of 2.6 t yr^{-1}). In this plot a different behaviour was observed in relation to the depth of the soil layer. In the first layer (0-20 cm) an increase from 1.56% to 2.11% was observed, while a slight decrease from 1.59% to 1.22% was recorded in the deeper layer (21-40 cm). In plots with *M. sativa*, a continuous increase of SOM (about 29.5%) was observed during four years, corresponding to an average of 7.4 t ha^{-1} of CO_2 stored. The benefit of the cultivation of perennial crops instead of tilled crops was highlighted in a plot managed only with perennial crops (*Miscanthus* from 1997 to 2001 and *M. arborea* from 2002 until today) with an average increase in the first 40 cm of soil, from 1.04% to 2.05% of SOM, corresponding to 6.8 t ha^{-1} of CO_2 stored.

Conservation of biodiversity

The flora biodiversity of the Mediterranean Basin 'biodiversity hotspot' is outstanding, with 15,000 to 25,000 species, 60% of which are unique to the region. These semi-natural agroecosystems are threatened by three main factors: the abandonment of husbandry, the intensification of agricultural practices and global warming. Moreover, the occurrence of recurrent fires in some Mediterranean areas due to the accumulation of flammable shrubby vegetation is of special concern from the environmental point of view, as it dramatically alters soil characteristics and impacts on the local flora and fauna communities (Rosa García *et al.*, 2010; Osoro *et al.*, 2012). All these disturbance factors affect biodiversity, which constitutes the most important stability factor of ecosystems and agroecosystems (Sala *et al.*, 2000; Duffy, 2002; Spehn *et al.*, 2005; Rockström *et al.*, 2009; Brussaard *et al.*, 2010; Fontaine, 2011). Rosa García *et al.* (2013) underlined that halting and reversing the decline of permanent pastures, including ligneous pastures, is one of the biggest challenges for the maintenance of European biodiversity and wider ecosystem services, including the Mediterranean areas, and that the development of proper management strategies is fundamental. The latest EU biodiversity targets support the maintenance of many semi-natural permanent pastures in farmlands, not only within Natura 2000, and the maintaining, enhancing and restoring of ecosystem services (EC, 2011).

There is growing evidence from several systems that important ecosystem processes, such as productivity and nutrient cycling, can be significantly related to the species richness of plants (Duffy, 2002). Sala *et al.* (2000) believe that the Mediterranean climate and grassland ecosystems are likely to experience the greatest proportional change in biodiversity because of the substantial influence of all drivers of biodiversity change. Spehn *et al.* (2005), analysing

the relationship between plant diversity and ecosystem functioning within the European BIODEPTH network of plant-diversity manipulation experiments, showed that altering biodiversity changes in the numbers and types of plant species and functional groups in experimental communities significantly affected all ecosystem processes examined over the investigated three-year period.

The consistent effects of species richness on multi-functionality over and above those of climate and abiotic factors highlight the importance of plant biodiversity as a driver of multifunctionality in drylands. Temporal stability of the ecosystem increases with diversity, despite a lower temporal stability of individual species, because of both portfolio (statistical averaging) and over-yielding effects. Scientific results indicate that the reliable, efficient and sustainable supply of some foods (e.g. livestock fodder), biofuels and ecosystem services can be enhanced by the use of biodiversity (Tillman *et al.*, 2006; Lüscher *et al.*, 2008).

Novel products from grassland (bioenergy and biorefining)

As reported by Rösch *et al.* (2009), in Central Europe an increasing portion of the grassland is no longer needed for feeding cattle and is now attractive for energy purposes. In southern Europe this situation does not usually occur under rainfed conditions, due to the scarcity of forage availability.

Owing to the high hemicellulose and cellulose and low lignin content, permanent grassland might be successfully used as feedstock for second-generation ethanol production. Generally, the more delayed the harvest the higher the structural polysaccharides (Prochnow *et al.*, 2013), which are the primary substrates for ethanol production. In a typical Mediterranean grassland, the hemicellulose content decreases when harvest is delayed from June to September, while cellulose content increases (Martillotti *et al.*, 1996). In order to calculate the theoretical ethanol yield (TEY) the equation reported in Scordia *et al.* (2014) may help to assess either the TEY from a dry feedstock tonne (kg ethanol DM t^{-1}) and the TEY per unit land area (L ethanol ha^{-1}). Overall TEY, summing up cellulose and hemicellulose content, might rise from 566.7 to 599.3 kg ethanol DM t^{-1} moving from June to September cut. The biomass yield of unsown permanent grassland in the Mediterranean area is in the range of 1.0 to 5.0 t DM ha^{-1}, leading to a minimum of 718.3 L of ethanol ha^{-1} to a maximum ethanol value of 3798.0 L ha^{-1}.

In Europe, the most investigated perennial grasses for energy purposes are switchgrass, reed canary grass, miscanthus and giant reed (Lewandowski *et al.*, 2003; Cosentino *et al.*, 2006; Cosentino *et al.*, 2007; Monti *et al.*, 2008, Scordia *et al.*, 2013). Actual Mediterranean constraints and predicted climate change now require the identification of native perennial grasses that are able to use natural resources efficiently (www.optimafp7.eu). In this regard, Copani *et al.* (2013) are evaluating the physiological and productive responses of some native perennial grasses widespread in semi-arid Mediterranean area, such as *Oryzopsis miliacea* (L.) Asch. & Schweinf, *Cymbopogon hirtus* L. Janchen, *Lygeum spartum* L.*, Sorghum halepense* L. (Pers.), or endemic in Sicily as *Saccharum spontaneum* L. *spp. aegyptiacum* (Willd.) Hackel*, for bioenergy purposes. *Saccharum* was the highest yielding species both at first and second year harvest (9.8 and 18 t DM ha^{-1}), while *Sorghum*, *Cymbopogon* and *Oryzopsis* showed no significant differences both at first and second year (2.7 and 5.5, 2.6 and 4.0, 3.5 and 3.6 t DM ha^{-1}, respectively). In the same environment, Cosentino *et al.* (2012) showed that *Saccharum* reached yields higher than 30.0 t DM ha^{-1} when 50% or 100% ETm restitution was applied in an older stand and indicated this species as potential energy crop for this environment. A similar activity is being carried out in Sardinia with the native *O. miliacea*, *Ampelodesmos mauritanica* Th. Dur. & Schinz. and *Hyparrhenia hirta* (L.) Stapf*, where these species are studied. From the preliminary results, *O. miliacea* seems the most promising species, showing high DMY and a favourable biomass allocation, mainly in tillers (Porqueddu *et al.*, 2014 [these proceedings]).

Previous experiments showed that *Miscanthus* needs irrigation water to achieve high yields in the Mediterranean area (27 t DM ha^{-1} with 100% ETm restitution) (Cosentino *et al.*, 2007). Results were corroborated by a long-term *Miscanthus* trial (17-years) grown in rainfed condition, which showed a marked yield reduction when rainfall decreased from 952 to 467 mm yr^{-1} (15.4 and 9.8 t DM ha^{-1}, respectively). On the other hand, giant reed (16-year-old stand) grown in a similar long-term experiment seems to be more drought resistant than *Miscanthus;* indeed, biomass reduction resulted more contained as drought increased (20.2 and 17.7 t DM ha^{-1}) (OPTIMA project, unpublished results).

Given the multiple functions that grassland and grassland landscapes can provide for society, their use as a source of energy feedstock has to be carefully evaluated. Grassland might need economic support for it to be comparable to other high yielding species dedicated to biomass production (Leible *et al.*, 2005). In this context, the use of grasslands for bioconversion or heating/energy production may be restricted to areas that cannot be ploughed, and in marginal environments in general after satisfying the requirements for livestock feeding (Peeters, 2009).

Future challenges and perspectives

The newly recognized multifunctional role of grasslands requires a renewal in grassland science, whose objectives should shift from the focus on the main function of grassland as a forage resource to a much broader concept of sustainable resource management involving environmental protection and conservation, livestock production and socio-economic development. This is especially true for the Mediterranean area, where land abandonment is a threat for grassland-based pastoral systems and the natural environment. The importance in soil protection and for carbon sequestration to prevent greenhouse gases emissions has been shown, as well as the possibility of bioenergy production from grassland as a renewable source of energy with low greenhouse emissions. Considering the importance of the Mediterranean basin as biodiversity hotspot, the opportunity to develop plant conservation strategies must be considered and risk maps at eco-regional scales should be used to inform stakeholders of grassland vulnerabilities, and to suggest management recommendations for ecologically significant areas expected to be sensitive to climate change. Despite their ecological, economic and social importance, grasslands still receive only limited scientific, political and media attention in relation to their conservation merits. This is mainly because they are widely perceived as bad and/or degraded lands suitable only for grazing, although in many Mediterranean marginal areas of Europe permanent grasslands are the basis of pastoral farming systems characterized by a low input management and the exploitation of local rustic breeds. The outcomes of these pastoral systems are farm products with special sensorial and nutritive qualities which are comparable to organic production. Therefore, the organization and full valorization of these products is desirable, as this can also play an important role in connecting rural and urban culture, and consequently rural people may be valued properly according to their importance for the actual society.

Future multidisciplinary investigations on particular grasslands types and plant species components related to the quality and value of livestock products are needed. At the same time, a successful development of a European seed industry for well-adapted Mediterranean grassland species is needed to make effective the selection activities carried out by several public research institutions. Finally, more efforts in on-farm experimentation and knowledge transfer to farmers are required, with a special focus on the correct incorporation and management of legumes and grass-legume mixture in agricultural systems, which is necessary for their full exploitation.

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Theme 1 'Climate change: mitigation and adaptation'

Theme 1 invited papers

Synergies between mitigation and adaptation to climate change in grassland-based farming systems

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Abstract

Climate change mitigation and adaptation have generally been considered in separate settings for both scientific and policy viewpoints. Recently, it has been stressed (e.g. by the latest IPCC reports) the importance to consider both mitigation and adaptation from land management together. To date, although there is already large amount of studies considering climate mitigation and adaptation in relation to grassland-based systems, there are no studies that analyse the potential synergies and tradeoffs for the main climate change mitigation and adaptation measures within the current European Policy context. This paper reviews which mitigation and adaptation measures interact with each other and how, and it explores the potential limitations and strengths of the different policy instruments that may have an effect in European grassland-based livestock systems.

Keywords: Mitigation, adaptation, resilience, climate change, grassland-based, livestock

Introduction

In the last IPCC report (AR5-WGIII: IPCC, 2014b), for the first time, most of the terrestrial land comprising agriculture, forestry and other land use (AFOLU) was considered altogether. Moreover, it was also highlighted in the AFOLU chapter (IPCC, 2014b) the importance to consider the systemic feedbacks and interactions between mitigation and adaptation options from land management (Separate sub-section: 11.5). In grassland-based systems, however, the potential interactions between mitigation and adaptation options, compared with forest or arable systems, have received much less attention and this has been reduced to changes in carbon (C) stocks and pasture productivity. Changes in biogeochemical cycles (mainly C and N) and water cycles are expected to exert large impacts on livestock productivity and N and C emissions from grassland-based systems (i.e. CO_2, CH_4 and N_2O). Climate change impacts on livestock will include effects of forage and feed quality and productivity, direct impacts of changes in temperature and water availability on animals, and indirectly through livestock disease increase (IPCC, 2014a). However, socio-economic changes are expected to have a still greater effect on mitigation and adaptation potentials (Schmidhuber and Tubiello, 2007).

Climate mitigation options in grassland systems mainly include practices that increase soil C stocks or can help to reduce GHG (greenhouse gases) emissions at the soil, feed, animal or manure management level. However, at a wider context, demand-side measures (e.g. human dietary changes, reducing losses and wastes in the agro-food chain) or substitution of fossil fuels by biomass can also play an important role in mitigating climate change. Mitigation options in grassland-based systems need also to be addressed for their potential impact on all other ecosystem/environmental services provided by grasslands for current and future scenarios, as climate regulation is just one of the services amongst a varied list (e.g. food

production). Mitigation and adaptation in grassland-based systems are closely integrated through a network of feedbacks, synergies and risk of trade-offs. Mitigation measures may also be vulnerable to climate change or there may be possible synergies and trade-offs between mitigation and adaptation options (IPCC, 2014b).

Many policies are directly (e.g. Kyoto Protocol) and indirectly (e.g. Common Agricultural Policy: CAP) affecting the potential success of implementing measures that both reduce GHG emissions and help to adapt grassland-based livestock systems to climate change. There is however a need for a more integrated and scientifically-based regulatory approach.

Although there are already many studies that have reviewed measures regarding grassland-based systems on GHG mitigation (e.g. Smith *et al*., 2007; Verge *et al*., 2007) or climate change adaptation (e.g. Bryan *et al*., 2009; Olesen *et al*., 2011; Tingem *et al*., 2009), only few sudies have assessed the benefits and trade-offs of their synergistic effects (e.g. Lal *et al*., 2011).

The main objective of this paper is to provide a high-level assessment of synergies and trade-offs for the main potential climate change mitigation and adaptation measures in grassland-based livestock systems within the current European Policy context.

Climate change mitigation now and in the future in grassland-based livestock systems

The main aim of the mitigation options in grassland-based systems is to reduce emissions of CH_4 or N_2O and/or to increase soil C storage, especially by soil as grasslands account for 75% of C in the terrestrial ecosystems (Lal, 2005; Dresner *et al*., 2007). Recently, more or less comprehensive reviews on GHG mitigation from grassland-based systems have been produced (e. g. Project ANIMALCHANGE: Van den Pol-van Dasselaar, 2012; UNEP, 2013; Havlík *et al*., 2014; Del Prado *et al*., 2013a). Nitrous oxide is formed in the soil through nitrification and denitrification (Wrage *et al*., 2001) and controlled by a number of site-specific factors, including soil moisture content (Del Prado *et al*., 2006), temperature (Dobbie *et al*., 2001) and also, management factors such as fertilizer (Cardenas *et al*., 2010) and management of soil organic matter content (Mosquera-Losada *et al*., 2011a) and grazing (Van den Pol-van Dasselaar *et al*., 2008). Carbon sequestration also depends on edaphoclimatic conditions (Theng *et al*., 1989), the presence of trees (Mosquera-Losada *et al*., 2011a) and the organic matter quality and quantity (Mosquera-Losada *et al*., 2011a and 2011b). Methane can be produced via enteric fermentation, which depends greatly on the level of feed intake, the quantity of energy consumed and feed composition, or can be produced at the manure management level, which increases with temperature, and with increased biodegradability of the manure (Monteny *et al*., 2006).

As highlighted in the last IPCC report on mitigation of climate change in the AFOLU sector, there is an emerging scientific activity on prediction of the likely impact of the climate change on the potential to reduce net GHG emissions (*i.e.* impacts on N_2O and CH_4 emissions and on the rates of C sequestration) in the AFOLU sector in general, and in grassland-based systems in particular. Mitigation options available today in the grassland-based farming sector may not be available or as effective with further global warming. Soil C storage has been shown to be vulnerable not only to climate change but more importantly, to changes in the disturbance regime, both natural and human-induced. Land use projections indicate large changes in land use and potentially this change will exacerbate the release of CO_2 from soils including grasslands. Increasing temperatures, when water is not limiting, are expected to accelerate soil organic matter (SOM) decomposition rates but result in an increase of C returns through plant residues considering the CO_2 fertiliser effect and the lengthening of the growing season (Bindi and Olesen, 2011). Increasing SOM will enhance soil C storage and may also increase above and belowground biomass production or at least improve yield stability (Pan *et al*., 2009). However, biological processes resulting in N_2O emissions (*i.e.* denitrification) could be

stimulated by greater SOM. Moreover, increased variability and higher frequency of extreme events will negatively impact soil C storage, by both decreasing production levels and enhancing soil C losses. At the manure level, GHG emission changes are expected in relation to temperatures and sometimes indirect effects driven by changes in the composition of the feed (e.g. digestibility).

Main climate change effects on European grassland-based livestock systems

During the last century, the climate in Europe has changed more than in other areas of the world (IPCC, 2007). Compared to the pre-industrial era, when the mean annual temperature increased by 0.8 °C globally, it increased by 1.2 °C in Europe. Based on theoretical models, a further increase of 1.0–5.5 °C is expected by the end of the twenty-first century (Christensen *et al.*, 2007). The increase in temperature has been most apparent in mountainous areas such as the Alps, which tend to have high biodiversity and where temperature increased by 2 °C during the twentieth century (EEA, 2009). This is twice the average temperature increase for the northern hemisphere. In addition, the quantity and distribution of precipitation have also changed in Europe during the twentieth century. Although there has been a 20% decrease in rainfall in southern Europe, there has been a 10–40% increase in rainfall in northern Europe. Furthermore, an increase in the frequency of extreme weather events is predicted across the European continent (EEA, 2008).

The most important impacts of climate change on grassland-based farming systems in Europe are expected to be through changes in pasture productivity and forage quality, therefore potentially affecting the duration of the grass growing season and the forage supply to ruminants. An example about how different intra-year temperature and precipitation regimes affect total and also seasonal distribution of pasture was found by Mosquera-Losada and González-Rodríguez (1998) in dairy systems. This paper highlights the importance of having flexible grazing systems, which affects annual and instantaneous stocking rate. The intensification of the hydrological cycle caused by more intensive rainfall and longer dry periods is expected to result in higher risks of soil erosion and nutrient leaching in currently wet temperate climates. Changes in precipitation patterns in drier areas will lead to higher dependency on stored soil moisture storage and seasonality for supporting grass growth. Elevated concentrations of CO_2 may also increase water use efficiency through reduced plant stomata aperture, but increase run-off risk through reduced plant transpiration, thus resulting in excess water at the land surface (Betts *et al.*, 2007). Biological and physical processes regulating nutrient cycling in grassland-based farming systems may actually be more sensitive to extreme events rather than changes in average climatic conditions. For Europe, an increased risk of low forage production in summer due to severe summer droughts events is expected to be offset by the appearance of new opportunities for forage production in other seasons due to warming effects. For southern latitudes, higher evapotranspiration rates will negatively affect grass yield and the period of grass growth will be shorter unless the grassland is irrigated. In general, poorer grass nutritional qualities, e.g. lower grass digestibility, can also be expected.

Potential synergies and tradeoffs between strategies to adapt to climate change and measures to mitigate GHG emissions

GHG emission mitigation choices may further enhance or reduce resilience to climate variability and change in terms of ecosystem goods and services provision, and thus influence the potential of grassland-based systems to adapt to climate change. climate change may affect climate adaptation and mitigation strategies through changes in feed supply, animal diet composition, animal and plant breeding, soil management, enhancement of floral biodiversity and via more resistant and resilient production systems against climate change (e.g. agroforestry systems).

Climate change affecting feed supply (grazing and forage)

Spring growth, provided that water resources for grass growth are available, and winter production may benefit from mild climate conditions. This can contribute to improvement in the farm´s degree of forage autonomy and security of livestock systems when facing more hazardous climate conditions (e.g. summer droughts) through the extension of the grazing season and the reduction of forage requirements (Graux *et al*., 2013). For example, forage resources usually stored for over-wintering livestock could be partially redistributed in summer to deal with increased risk of forage deficits (Graux *et al*., 2013). However, for southern latitudes and dates getting closer to the XXII century (e.g. UK: Del Prado *et al*., 2009) the projections suggest that grazing activity will be constrained due to too high temperatures and excessive drought in Europe. Extending grazing seasons by e.g. the presence of shelter/shade belts of trees would reduce the wind speed and therefore evapotranspiration (ETP). The presence of trees at low density would also increase the duration of the growing season due to their presence, which may partly reduce GHG emissions (Tackas and Frank, 2009) through improving soil N recovery by trees, but may also become hot-spots for N_2O from overlapping urine patches, and soils could become eroded due to the action of hooves in camping areas used by livestock for shelter/shade. Extending the grazing season in some cases may also be limited by the bearing capacity of the soil driven by good soil structure degradation (e.g. poaching caused by trampling cattle or/and severe summer droughts, *etc*.) and therefore, it may, in some cases be impractical. Hence, avoiding compaction by traffic, tillage (Pinto *et al*., 2004) and grazing livestock (De Klein and Ledgard, 2005) may help to maintain grasslands in good conditions and also to reduce N_2O emissions.
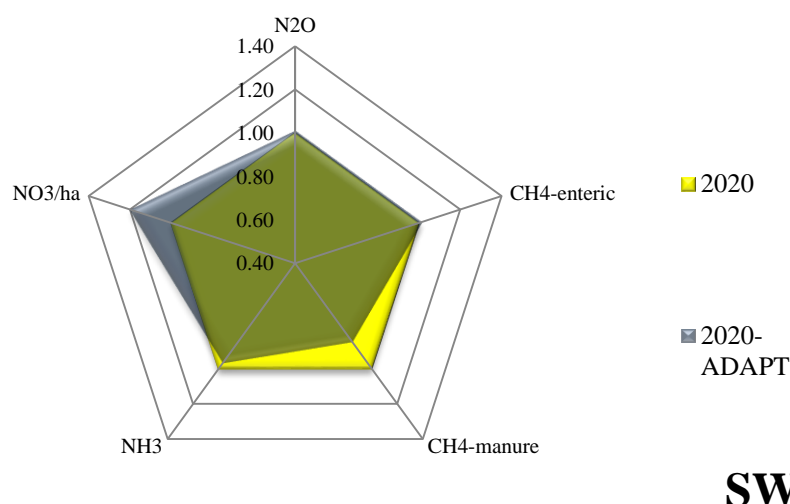
Figure 1. Comparison between adapted (extending one month grazing) and un-adapted typical dairy farms in the south west England (2020) for GHG, NO_3^- leaching and NH_3 emissions. Values for the adapted scenario <1 indicate a reduction in emissions (adapted from Misselbrook *et al*., 2013).

Poorer grass nutritional qualities, e.g. lower grass digestibility, will lead to higher CH_4 emissions from enteric fermentation of cattle (Hart *et al*., 2009). Although if lower forage quality would mean reduced livestock 'yields' and/or quality then market reaction would be to import feeds. For those systems extending the grazing season (e.g. Figure 1: UK: Misselbrook *et al*., 2013), smaller volumes of manure storage could lead to a reduction in CH_4 emissions from manure handling. However, the rise in average and extreme temperatures, should the frequency of manure removal from the storage remain unchanged or no additional structural measures are implemented to either lower the manure storage temperature or to aerate the slurry, could increase the amount of CH_4 per kg of volatile solid of manure and therefore, lead to similar total CH_4 emissions from manure management. Moreover, even if the CH_4 emissions

from manure management were smaller, this value (in CO_2 equivalents) would be partially offset or accentuated by an increase in N_2O emissions promoted by the increase in grazing activity.

Climate change affecting feed supply (purchased feed and different crop rotations)

Changes in grassland productivity will affect either animal productivity or the amount of purchased feed required (Mosquera-Losada and González-Rodríguez, 1998). For semi-arid regions (e.g. south of Europe), a reduction in annual grass productivity will lead to lower animal productivity or will have to be compensated with a larger share of imported feedstock with associated monetary and environmental costs, which may translate into a potential loss of resilience in grassland-based livestock systems. In some of these regions tree presence to feed animals with acorns, could supply part of these needs (Moreno and Pulido, 2009).

An increase in the establishment of rotations best suited to the area or crop rotations with legumes annual crops (Bryan *et al*., 2011) may also occur as an adaptation strategy. Some crops that currently grow mostly in southern Europe will become more suitable further north or in higher altitudes areas in the South. For example, forage maize, may become more common across in the boreal regions of Europe. Maize forage, however, tends to make the management system less flexible to inter-annual temperature/precipitation variations.

Moreover, maize area cannot be used for grazing during the summer or autumn if no grass is available during this period. In contrast, grass areas can be open or harvested for silage if a restriction or an excess of grass production happens (Mosquera-Losada and González-Rodríguez, 1998). At the animal level, forage maize animal intake is generally promoted at the expense of grass due to a better balance between protein intake and soluble carbohydrates (e.g. through increasing starch concentration in the diet), which additionally may help to increase animal energy use efficiency and decrease CH_4 emissions per kg DM intake. However, this CH_4 reduction may be offset by larger N_2O emission losses and a larger CO_2 release of converting some grassland into arable land (Vellinga and Hoving, 2011).

Conversely, converting crops to pasture has been found to reduce N_2O emissions (Eagle *et al*., 2012) and also contribute to sequester soil C, especially in the first years after conversion. Leguminous species are well adapted to future conditions of climate change (Kreyling *et al*., 2012) considering that their optimum temperature is higher than non-leguminous crops and that they also have more positive responses to elevated concentrations of CO_2 (Soussana and Lüscher, 2007) than non-legume species. In a situation with a larger share of mixed legume/grass pastures, in addition to presenting climate adaptive advantages over conventional pastures, these systems have lower requirements for N fertilizer through the use of biological N fixation of nodules on the roots of legumes, which would lead to energy savings and GHG emissions reductions from both fertilizer production and use (Del Prado *et al*., 2011; Zhang *et al*., 2013).

Climate change affecting feed supply (use of by-products and alternative forages)

Different by-products from agricultural, forestry, agro-industry and bioenergy activities can also be used for feeding ruminants as an adaptive response to forage supply seasonal constraints. Rinne *et al*. (2012) reviewed different by-products (e.g. camelina meal, tomato pomace) that are currently underutilized but that could potentially be used as feed for low input and organic dairy production systems. Those practices are currently used as part of some livestock systems at a regional level (Correal *et al*., 2009). These by-products vary in their geographical availability, nutritional value, their effect on rumen CH_4 and N excretion (*i.e.* effect on GHG mitigation) and have logistic-related challenges. Environmentally speaking (e.g. GHG intensity), the use of some of these by-products as animal feed may not always be the best option in comparison with their use in bioenergy or for soil improvement. In this sense,

removal of crop residues from cropping systems for use in bioenergy, if this means that soil C contents are being depleted (e.g. straw: Liu *et al*., 2014), will bring large risks of negative impacts on adaptation measures and potentially, small or negligible positive effects on the reduction of net GHG emissions. Mitigation and adaptation conflicts may therefore appear as one chooses a particular use of the by-product or another.

Other alternative forage supply may include tree leaves and shrubs, particularly in small-scale livestock farms with dry to semi-arid climates. Such species can alleviate feed shortages, or even fill feed gaps in the winter and especially in the summer, when grassland growth is limited or dormant due to unfavourable weather conditions (Papanastasis *et al*., 2008). Although some species have leaves with a low content of CP and a high content of fiber and contain high levels of secondary compounds such as tannins, alkaloids, saponins and oxalates which reduce the nutritive value of poor-quality diets, some of these compounds (e.g. condense tannins), when improved temperate forages are fed, can also have substantial benefits for ruminant productivity (*i.e*. reducing CH_4) and health (Waghorn and McNabb, 2003). Moreover, there are also other species (e.g. *Morus alba*, *Fraxinus excelsior*, *Betula alba*) whose young leaves are rich sources of protein and fibre and generally used in the past to feed animals before modern techniques like fertilizer were used.

Changes in fertiliser management, diet and genetics to increase N use efficiency

Manipulating the diet (e.g. feeding nitrification inhibitors: Ledgard *et al*., 2008 or salt supplementation) during the grazing period has also been proposed as a means to reduce N_2O emissions. Improving fertiliser efficiency, optimising methods, timing and rates of applications (Brown *et al*., 2005), using NH_4^+-based fertilisers rather than nitrate-based ones (e.g. Dobbie and Smith, 2003) and employing nitrification chemical inhibitors (e.g. Zaman *et al*., 2009) may also have a role in both mitigation (i.e. reduction of direct and indirect soil N_2O emissions) and adaptation (through a better N use efficiency at the soil-plant level).

New traits in animals and grasses may also assist farmers to both mitigate and adapt to climate change. Del Prado and Scholefield (2008), for example, using a farm modelling approach, evaluated the scope for different animal and plant genetic traits, some existing and other theoretical, to help reduce GHG emissions on UK dairy farms. More efficient animals in utilising N (Alford *et al*., 2006) have also been proposed to decrease the impact of urinary N during grazing. Some of the traits, e.g. improved N use efficiency in grasses (e.g. high sugar grasses: Wilkins *et al*., 2000) could actually be both potentially useful for climate mitigation and may also promote Climate adaptation as they may reduce GHG emissions from urine-related N_2O emissions and improve the quality of the forage, which may be beneficial in future scenarios where climate has a detrimental effect on grass nutritional properties.

Soil management, plant biodiversity and new plant breeds to improve system resilience against environmental stress conditions and prevent soil erosion

Other strategies to both mitigate and adapt to climate change may involve management practices that target directly to the soil, both improving the capacity to store water and to prevent soil erosion. By increasing the ability of soils to hold soil moisture and to better withstand erosion by enriching biodiversity through more diversified cropping systems, grassland systems will be able to sequester more soil C and also to better resist extreme events such as droughts and /or floods, both of which are projected to increase in frequency and severity in future warming climates (Rosenzweig and Tubiello, 2007).

The measures for the conservation of soil moisture may also include changes in tillage practices. Reduced tillage, for example, increases the resilience to climate change through improved soil fertility and increased capacity for water retention in the soil. This improvement is expected in the long-term productivity potential when tillage is reduced (Olesen *et al*., 2011).

The reduced tillage at pasture reseeding promotes C sequestration and preservation in pastures and is considered to be more effective under conditions of water deficit (Alvaro -Fuentes *et al.*, 2011). It leads also to significant savings in CO_2 emissions produced by machinery. However, the impact on N_2O emissions under different conditions is unclear (Estavillo *et al.*, 2002; Pinto *et al.*, 2004). Nitrous oxide emissions appear to be strongly influenced by soil water content immediately after nitrogen fertilization (Del Prado *et al.*, 2006). In view of this dominant effect of a particular soil moisture level coinciding with tillage and fertilization, it is key to find the best timing for the renewal of pasture. Velthof *et al.* (2010), for example, considering average Dutch climatic season conditions, suggest that this pasture renewal should take place in spring rather than fall because Dutch autumn, compared with spring is generally wetter and N uptake by the reseeded grass is lower. The effect of reduced tillage has also been observed by increasing the periods between which a pasture is renewed. Vellinga *et al.* (2004), for example, found that although tillage increased N_2O and CO_2 in the intensively managed pastures in the studied year, in the long run, the renovation of pastures was more important to prevent deterioration in pasture quality and thus, to prevent from soil loss and large productivity losses. For areas which are subject to severe or extremely severe environmental stress conditions the establishment of a community of pastures formed by species that ensure ecological stability, both in ecosystem resistance and resilience, is key as an adaptation measure to climate change (Volaire *et al* ., 2014). Additionally the species composition of the pasture is expected to undergo changes, as for example, warming will favour C4 species over C3 species (Howden *et al.*, 2008). Biodiversity should act as a safeguard of ecosystem functioning, thus promoting a more stable ecosystem to avoid fluctuations arising from adverse climatic fluctuations (Volaire *et al.*, 2014). Promoting biodiversity could also have an effect on the mitigation potential of pastures and in some occasions of rumen methane. Considering that N remains one of the main elements that determines the diversity of plants, the application of less fertilizer should be a requirement to increase diversity in different floral species in grasslands (Mountford *et al.*, 1993). This reduced input fertilizer would be necessarily associated with lower emissions of N_2O per ha and potentially a greater amount of C accumulated in the soil.

New grass breeds have already been tested to improve water use efficiency. For example, McLeod *et al.* (2013) tested in the UK a novel grass *Festulolium* hybrid capable to reduce runoff by 40-50% compared to a leading UK nationally recommended L. *perenne* cultivar and F. *pratensis* over a two year field experiment. The rapid growth and turnover of roots in the hybrids resulted in greater soil water storage capacity in the plots with observed lower rainfall runoff. This may, in turn, have significant effects on N_2O emissions and soil C storage.

Agroforestry systems

Agroforestry is a well-founded example of mitigation and adaptation synergy (e.g. IPCC, 2014b; EU forest strategy: EU, 2013) since trees planted and grassland soils sequester C and tree and grassland products provide livelihood to communities, especially during drought years (Verchot *et al.*, 2007). Agroforestry in general and silvopastoral systems in particular lead to greater resilience to climate change due to improved soil conditions and management efficiency in water use (Kumar *et al.*, 2011). Its characteristics are able to reduce evapotranspiration and thus improve the maintainability of soil water (Tackas and Frank, 2009). These practices also have a great potential to offset GHG emissions through the sequestration of C in soil and tree biomass and avoiding the release of NO_3 leaching (indirect N_2O emissions) (Rigueiro *et al.*, 2009). Moreover, these systems also improve the N use efficiency of the system and offer large resilience against climate change stress conditions through the reduction of temperature of the system (Rigueiro *et al.*, 2009). It can also help reduce erosion of adjacent fields handled more intensely (Verge *et al.*, 2007).

Policy implications

Climate mitigation policies and measures may exhibit synergies and risk trade-offs with climate adaptation (Bates *et al*., 2008). However, policies of mitigation and adaptation are often being considered in separate settings, resulting in potential conflicts. An integrated adaptation and mitigation framework is important to ensure that trade-offs between the two are minimized and synergies encouraged (Wreford *et al*., 2010). However, this is not easy as mitigation and adaptation may occur simultaneously, but differ in their spatial, timing and geographical characteristics (Smith and Olesen, 2010).

Amongst the number of policies affecting climate change mitigation and adaptation in grassland-based systems in Europe, the newly reformed EU Common Agricultural Policy (CAP), in principle, has made a decisive move towards promoting a greener and climatically friendlier EU agricultural sector. The new CAP has introduced direct payments associated with different practices that, in some cases, are expected to enhance GHG mitigation and adaptation to climate change. Specifically, new payments within Pillar I associated with the diversification of crop rotations, maintaining permanent pastures and ensuring Ecological Focus Areas should be targeting, in part, climate-friendly or climate-smart agriculture. Permanent pasture maintenance is an important way to prevent N emissions through avoiding plough management and conversion of permanent grasslands into arable lands (EU Regulation 1307/2013). Leguminous species are mentioned explicitly in the areas of ecological interest (N-fixing species) but there is no special plan for their promotion. Other practices, such as those mentioned in previous sections, grazing, for example, is encouraged directly through the support of agroforestry systems and forests with fire risk areas (through the Rural Development Programme (Pilar II)), avoiding huge amounts of C release and through cross-compliance via for example promotion of good standards for animal welfare. Floristic biodiversity should also be encouraged but are not explicitly mentioned within the new PAC to safeguard ecosystem functioning against adverse climatic fluctuations.

The replacement of permanent grasslands by forage maize is no longer allowed by the CAP as penalties are included in the last CAP if destruction above 5% is present. The new CAP, however, does not explicitly address the worrying import of feed in grassland-based intensive systems. In fact, in some countries, this is still indirectly encouraged through additional payments to more intensive systems. The CAP has been blamed for distortion of global markets in this sense. Khatun (2012) points at the absence of tariffs for animal feed as a key driver for fueling EU cheap imports of animal feed from Latin America and consequently, for the effect on land use, land use change, and forestry (LULUCF) outside of the EU and, thereby preventing from a huge potential for mitigating climate change by reducing emissions from deforestation and forest degradation (REDD+ programme) outside Europe. Policies, hence, can create both positive and perverse incentives for mitigation or adaptation (Wreford *et al*., 2010). A number of recent studies (e.g. Lassaletta *et al*., in press in Spain) suggest that many European Countries, either assisted by specific regulations (e.g. Kyoto, CAP) or fuelled by market pressures, are displacing large amounts of GHG emissions from their national primary sectors (e.g. grassland-based farming) to other countries via agricultural goods importing. For example, cattle farming in Europe, whose feed system was traditionally based mainly on-farm forage (e.g. grass) production, in the recent decades has shift to heavily depend on cheap imported protein (e.g. soybean) from South America, resulting in a reduction of GHG emissions in the European GHG inventories but more than offsetting this potential mitigation by a consequential increase of GHG emissions by mainly land use change in South America. Much of these emissions are produced in non- Annex B countries and consequently, C leakage is being produced in Europe. Displacing agricultural productivity may indeed be an adaptation choice for countries, but this is certainly against securing Food Sovereignty and therefore, this jeopardizes the future resilience of the European food system. For example, if the conversion of annual crops to pasture is accompanied by a demand to grow annual crops outside Europe, this would not

represent a net mitigation but merely a shift in emissions and, in some cases, this would be an example of C and / or N_2O leakage.

Furthermore, a large part of the mitigation potential of grasslands is also subject to challenges in relation to effectiveness over different time-scales. For example, whereas certain types of mitigation activities (e.g. N_2O reduction from reduced N fertilization, CH_4 reduction in the rumen through animal diet changes, bioenergy) are effectively permanent since the emissions, once avoided, cannot be re-emitted (IPCC, 2014b), some activities that helped to sequester C (e.g. reducing tillage), can be reversible and non-permanent. Moreover, some of these practices to sequester soil C may also be constrained due to the saturation of grassland soils to sequester C indefinitely. Therefore protecting the large C stocks in grasslands should be an important management and policy target, rather than necessarily trying to increase the C stocks (Smith, in press) since it is easier and faster for soils to lose C that it is for them to gain C (Johnson *et al.*, 2009).

Mitigation options for any of the GHG gases must also be tailored to the specific soil, climatic and production system conditions (Bustamante *et al.*, in press). There will be very few strategies that are universally applicable for all systems and under any climatic circumstances. All mitigation options certainly affect and are affected by the cycles of C and N. Nitrogen and C cycles are also currently decoupled for most intensive grassland systems (Soussana and Lemaire, 2014), these systems release by ruminants bound-C digestible as CO_2 and CH_4, and return digestible N in high concentrations (urine patches). The coupling / decoupling of C and N makes an added difficulty to analyze the effectiveness of mitigating measures as sometimes some of the measures that increase soil C storage, for example, addition of manure, can also increase losses of N_2O by increasing soluble C in the system. In contrast, measures that promote the reduction of N_2O can cause a net loss of C from the system through increased soil respiration (Scholefield *et al.*, 2005). Moreover, some of the mitigation methods lead to pollution swapping (e.g. NH_3 volatilization , leaching of NO_3^-), and losses in biological diversity and / or productivity (Del Prado and Scholefield, 2008), and also can cause numerous interactions between mitigation measures so that their effect in the case of using multiple measures simultaneously are not necessarily additive (Del Prado *et al.*, 2010).

Also, the reference unit to which GHG emissions relate within the CAP is commonly the forage area, which may not, in some cases, coincide with the preferred reference unit used by the agroindustry (C footprint or GHG per unit of product). The emphasis therefore seems to have been diverted from what the consumers and markets dynamics are essentially promoting. Preferably, one should consider more than one reference unit or functional unit (e.g. per hectare and per unit of output) at the same time to avoid conflicts of interpretation about what is true / false mitigation (Del Prado *et al.*, 2010). Agroindustry generally uses the Life Cycle Assessment (LCA) as the methodology choice in order to report GHG emissions from the full cycle of the production of a food. A key element still unsolved is the way LCA assigns different amounts of GHG emissions to different goods according to its market-based value. Given a specific policy context, the farmer may choose among the most cost-effective and easier-to-adopt options. Ecosystem services which currently have no market value may become valuable also in monetary terms in the future. Some farmers may, therefore, in the future also seek to maximize the ecosystem service value. Alternative methodologies are already suggesting that, for products that are produced through extensive and in some cases greener conditions, these emissions should be split according to not only market but non-market (e.g. ecosystem services) values (Ripoll-Bosch *et al.*, 2013) as well.

An important issue that may not be reflected in the new CAP and in other policies is the alarming growth tendency of feeding ruminants (e.g. dairy cattle: Del Prado *et al.*, 2013b) with a greater amount of feed ingredients which could be used directly in the human food chain (e.g. cereals) (Eisler *et al.*, 2014). This relates very significantly to the potential competitive

advantage that pasture-based livestock (ruminants generally are able to use low-quality plant biomass and that is inedible to humans) might have over another livestock (e.g. monogastric animals). Policies therefore should be useful to overturn this trend.

Additionally, non-climate policies and regulations are already in place for other environmental issues (e.g. water quality, NH_3) and have consistently assisted in reducing GHG emissions from the agricultural sector (e.g. EU: Velthof *et al*., 2014). Nitrate leaching losses, however, are expected to increase for numerous areas that are already constrained in their nutrient use by the EU Nitrates Directive (Anon, 1991) in Europe and for feed commonly used in animal diets, for example wheat (Olesen *et al*., 2007). This increase in NO_3^- leaching may trigger more stringent regulations and hence affect animal productivity and GHG emissions, which may challenge climate change adaptation also from a policy perspective. Research-oriented policies should and already have a role, for example, in encouraging the study of new grass varieties that can better adapt to climate change and also present properties that can increase the efficiency of use of nutrients and energy in the soil-plant-animal system.

It is therefore imperative that all the policies, from the local to the global levels, are appropriately integrated with the policies relating to climate change, bioenergy, food, waste, research and health in order to promote a net reduction of GHG from the standpoint not only of production (supply) but also of demand in order to avoid possible market distortions and maladaptation practices at all levels.

Acknowledgements

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The role of grassland in mitigating climate change

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Abstract

Grassland management has a large potential to mitigate livestock greenhouse gas (GHG) emissions at a low (or even negative) cost, by combining a moderate intensification and the restoration of degraded pastures. A synthesis of eddy flux covariance data shows, on average 213 site years, a mean net carbon storage (*NCS*) equal to 76 ± 11 gC m^{-2} yr^{-1} indicating a significant carbon sequestration in European grasslands. Through statistical modelling we show that this carbon sink activity is largely controlled by climatic and by management factors, with additional drivers from soil and vegetation types. Simple calculations show that pasture carbon neutrality (in CO_2 equivalents, accounting for enteric CH_4 and soil N_2O emissions) can be obtained below a critical stocking rate (*SR**). When climate is optimal for grassland canopy photosynthesis (10 °C and 1200 mm yr^{-1}), *SR** equals 0.85 and 1.2 LSU ha^{-1} over a 200 days grazing season without and with manure application, respectively. A low herbage use efficiency (ratio of intake to above-ground net primary productivity) comprised between 0.2 and 0.4 without and with manure application, respectively, is required to reach SR*. Suboptimal climate conditions lead to lower *SR** and herbage use efficiency values without manure application, while manure supply moderates this decline. In contrast to manure application, mineral N fertilizer supply leads to minor changes in *SR** values. Climate change affects the grassland carbon sink which is not permanent. A short-term change in temperature and precipitation has large implications for the GHG balance of temperate pastures. An exponential rise in GHG emissions per head is simulated with warming and a decline in precipitation affecting a pasture managed at the critical stocking rate under the current climate. The implications of these findings for grassland management are discussed.

Keywords: Climate change; Pasture; Livestock; Carbon; Greenhouse Gas; Soil.

Introduction

Since the industrial revolution, cumulated anthropogenic CO_2 emissions to the atmosphere reached 545 ± 85 Gigatons C (1 Gt C = 10^9 metric tons C). Land use change (including deforestation, afforestation and reforestation) contributed to one third of this amount. About half of the anthropogenic emissions since 1750 was removed from the atmosphere by sinks, and stored in the natural carbon cycle reservoirs. Vegetation biomass and soils not affected by land use change and land degradation stored 150 ± 90 Gt C and the ocean reservoir stored approximately the same amount (IPCC, 2013).

The current mean global temperature is slightly above the temperature range experienced during the Holocene, which has seen the onset and expansion of agriculture since ca. 10,000 yrs BP (Marcott *et al.*, 2013). By the end of the 21st century, the biosphere will experience unchartered conditions with a temperature rise between 1.5 and 4.5 °C compared to 1980-1999 and CO_2 concentrations in the range 450-1000 ppm (IPCC, 2013).

The entire agriculture, forestry and land use sector (AFOLU) contributes directly to 24 percent of total anthropogenic emissions, a value which has declined since 2005 given the reduction in tropical deforestation and in the intensity of GHG emissions per unit of agricultural production (IPCC, 2014). In a separate assessment, the FAO estimated greenhouse gas (GHG) emissions from livestock supply chains (from land use for feed production to the processing and transport of animal products) to represent globally 7.1 Gigatons of CO_2 equivalents or 14.5 percent of

global anthropogenic GHG emissions. The main sources of emissions identified by the FAO are related primarily to the production and processing of animal feed: this corresponds to 45 percent of total emissions, 9 of which are related to the expansion of grazing and crop areas at the expense of forests. Next comes methane emissions from the digestive process in ruminant animals (39 percent), followed by emissions from manure, at 10 percent. The remainder comes from the processing and transportation of animal products (FAO, 2013).

The grassland biome covers about one-quarter of the earth's land area (Ojima *et al.*, 1993). Except within eco-geographical regions where vegetation is maintained by climate and soil factors at herbaceous stage, most of the grasslands around the world are the result of livestock management avoiding encroachment by shrubs and trees (Lauenroth, 1979; Lemaire *et al.*, 2005). At the global scale, grasslands were estimated to be a net C sink of about 0.5 PgC per year (Scurlock and Hall, 1998), but with considerable uncertainty.

Grassland ecosystems hold large C reserves, mostly in soil organic matter. Historically, some of these soils have lost one-half to two-thirds of the original top soil organic carbon (SOC) pool with a cumulative loss of 3–4 tons C/ha. Especially in drylands, the depletion of soil C is accentuated by soil degradation and exacerbated by land misuse and soil mismanagement (Lal, 2004). Restoration of degraded lands and grazing land management have been shown to be key options for GHG mitigation in the AFOLU sector (IPCC, 2014) and the global soil organic carbon sequestration potential is estimated to be 0.01 to 0.3 Gt C/year for permanent pastures, which could potentially offset up to 4% of the global GHG emissions (Lal, 2004).

Follett and Schuman (2005) reviewed grazing land contributions to C sequestration worldwide using 19 regions. A positive relationship was found, on average, between the C sequestration rate and the animal stocking density, which is an indicator of the pasture primary productivity. Based on this relationship, they estimate a 200 Megatons SOC sequestration per year on 3.5 billion ha of permanent pasture worldwide. Using national grassland resource dataset and NDVI (Normalized Difference Vegetation Index) time series data, Piao *et al.* (2009) estimated that C stocks of China's grasslands increased over the past two decades by 117 and 101 g C/m^2 per year.

In Europe, Schulze *et al.* (2009) inferred a net C sink in grasslands of 57±34 gCm^{-2} yr^{-1} from a small sample of flux tower net CO_2 exchange measurements, completed by C imports/exports at each site to estimate the carbon balance. When accounting for emissions of non- CO_2 greenhouse gases (GHGs) such as methane (CH_4) from grazing animals and nitrous oxide from soil nitrification/ denitrification, the European grasslands were estimated to be nearly neutral for their radiative forcing, with a net balance of −14±18 g CO_2-Ceq m^{-2} y^{-1} (Schulze *et al.*, 2010). Therefore, non CO_2 emissions associated to grassland management by herbivores tend to offset the carbon sink which is currently observed in European grasslands.

After presenting the grassland carbon cycle, we review the evidence for a carbon sink in European grasslands and address drivers and trade-offs with non CO_2 emissions. Finally we discuss the risks of soil carbon losses associated to an increased climatic variability.

1. The carbon cycle in managed grasslands

While there has been steady C accumulation in soils of many ecosystems over millennia (Schlesinger, 1990), it is usually thought that soil C accumulation capacity is limited (Six *et al.*, 2002). Therefore, in a steady-state, non-disturbed soils should have attained C balance after several centuries (Lal, 2004). However, net primary productivity and soil respiration are currently affected by climate change in most regions of the world (Nemani *et al.*, 2003), which implies that soil C stocks are unlikely to have reached equilibrium (Soussana *et al.*, 2010c). The potential for sequestrating C in deep soil layers is also considered large (long residence time), but owing to the low influx of C within these horizons this process remains slow. The process-based ORCHIDEE-GM model was used to simulate the net carbon balance of

grasslands since 1900. Simulations show an increase in grassland carbon stocks over the last decades, with half of this effect attributed to changes in grassland management (reduction in livestock numbers) and the remainder explained by warming and rising atmospheric CO_2 which have both contributed to increase grassland productivity (Chang *et al.*, 2014).

Soil C sequestration is reversible and C can be rapidly lost through a number of processes such as soil disturbance, vegetation degradation, fire, erosion, nutrient shortage and water deficit. Therefore, agricultural practices like ploughing, which mix soil layers and break soil aggregates, accelerate top soil organic C decomposition (Paustian *et al.*, 1998, Conant *et al.*, 2001). Changes in SOC through time are non-linear after a change in land use or in grassland management. A simple two parameters exponential model has been used to estimate the magnitude of the soil C stock changes, showing that C is lost more rapidly than it is gained after a change in land use (Soussana *et al.*, 2004). As a result of periodic tillage and re-sowing, short-duration grasslands tend to have a potential for soil C storage intermediate between crops and permanent grasslands. Part of the additional C stored in the soil during the grassland phase is released when the grassland is ploughed up. The mean C storage increases in line with prolonging the lifespan of covers, that is, less frequent ploughing (Soussana *et al.*, 2004).

Under intensive grazing, up to 60% of the above-ground dry-matter production is ingested by domestic herbivores (Lemaire and Chapman, 1996). However, this percentage can be much lower under extensive grazing. The largest part of the ingested C is digestible and, hence, is respired shortly after intake. The non-digestible C (25 to 40% of the intake according to the digestibility of the grazed herbage) is returned to the pasture in excreta (mainly as faeces). The nature, frequency and intensity of disturbance play a key role in the C balance of grasslands. In a cutting regime, more than 80% of the above-ground primary production is harvested and exported as hay or silage, but part of these C exports may be compensated by organic C imports through farm manure and slurry application. Off-site C sequestration occurs whenever more manure C is produced by then returned to a grassland plot. The sum of on- and off-site C sequestration reached 129, 98 and 71 g C/m2 per year for grazed, cut and mixed European grasslands on mineral soils, respectively, however with high uncertainty (Soussana *et al.*, 2010c).

Long-term field observations show that even when plant material is incorporated in large amounts, the soil C content does not necessarily increase (Ammann *et al.*, 2007). Recent results suggest that N-deficiency may lead to a soil C loss though competition between SOC decomposing and storing microbes (Fontaine and Barot, 2005). Accordingly, long-term C storage in terrestrial ecosystems depends also on the ability to sequester nutrients, which explains the lower C sequestration in grasslands of low productivity and fertility (Franzluebbers *et al.*, 2007). Temperate grasslands have often been intensified by combinations of (i) an increased primary production through an improvement of the N-P-K status of vegetation, (ii) an increased stocking density for converting more efficiently herbage production into animal products, (iii) sowing, or over-sowing, of improved grass and legume species.

Intensification has three contrasting effects for the carbon cycle of grasslands (Soussana and Lemaire, 2014): first, an increase in the net primary productivity; second, a decline in the amounts of organic carbon returned to the soil (Soussana *et al.*, 2007); third, a possible decline in the turnover of soil organic matter when nutrients are in ample supply for soil microbes (reduced priming effect, Fontaine *et al.*, 2007; 2011). Depending on the balance of these effects, the impacts on the soil carbon balance may vary. Grassland intensification also leads to increased emissions of N_2O from fertilizers and biological N fixation, and to increased methane emissions from enteric fermentation. In comparison to an unmanaged control pasture, doubling the animal stocking density and supplying mineral N fertilizers led to increased net GHG emissions per unit area at an upland permanent pasture site in France (Allard *et al.*, 2007).

However, during dry years, the moderately intensive grassland was more resilient in terms of carbon storage, emitted less GHGs, and provided increased cattle liveweight gains (Klumpp *et al.*, 2011). Therefore, a moderate intensification of permanent pastures could provide an interesting combination of mitigation and adaptation.

Management practices that enhance C sequestration in temperate grasslands were summarized in several reports (*e.g.* Soussana *et al.*, 2004, 2010, Pellerin *et al.*, 2013):

- Grazing management (extension of grazing season, strip grazing, *etc*) systems that maximizes production, and increases carbon inputs and sequester carbon, while reducing GHG emissions,
- Sowing improved species can lead to increased production through species that are better adapted to local climate, more resilient to grazing, more resistant to drought and able to enhance soil fertility (*e.g.* N-fixing crops),
- Direct inputs of water, fertilizer and organic matter to enhance water and N balances, plant productivity and carbon inputs. However, inputs of water, N and organic matter all tend to require energy and can each enhance fluxes of N_2O, which are likely to offset carbon sequestration gains,
- Restoring degraded lands enhances production in areas with low productivity, increasing carbon inputs and sequestering soil organic carbon,
- Including grass in the rotation cycle on arable lands can increase production return organic matter (when grazed as a forage crop), and reduce disturbance to the soil through tillage. Thus, integrating grasses into crop rotations can enhance carbon inputs and reduce decomposition losses of carbon, with benefits for carbon sequestration.

2. Methods for assessing the carbon balance of a grassland

Two methods can be used for measuring the carbon balance of a grassland field: direct measurements of soil organic carbon stock change; carbon flux measurements allowing calculation the carbon balance. We briefly review below these two methods.

Soil organic carbon stocks

A number of studies have analysed effects of grassland and rangeland management on SOC stocks. Most studies concern only the top-soil (*e.g.* 0 to 30 cm), although C sequestration or loss may also occur in deeper soil layers (Fontaine *et al.*, 2007). It is often assumed that impacts of management are greatest at the surface and decline with depth in the profile (Ogle *et al.*, 2004). The uncertainties concerning the estimated values of C storage or release after a change in grassland management are still very high (estimated at 25 g C/m2 per year).

Data from the National Soil Inventory of England and Wales obtained between 1978 and 2003 (Bellamy *et al.*, 2005) show that C was lost from most top soils across England and Wales over the survey period. Nevertheless, rotational grasslands gained C at a rate of ca. 10 g C/m^2 per year. The Countryside Surveys of Great Britain are ecological assessments in UK that have taken place since 1978 (Firbank *et al.*, 2003). In this survey, significant increases in soil C concentration, ranging from 0.2 to 2.1 g/kg per year, were observed in both fertile and infertile grasslands (CLIMSOIL, 2008). In Belgium, grasslands were reported either to be sequestering C in soils at rates of 22 or 44 g C/m^2 per year (Lettens *et al.*, 2005a; Goidts and van Wesemael, 2007, respectively), or losing C at 90 g C/m^2 per year on podzolic, clayey and loam soils (Lettens *et al.*, 2005b). However, soil bulk density was estimated from pedo-transfer functions in these studies, which adds to the uncertainty, as a small change in bulk density can result in a large change in stock of SOC (Smith *et al.*, 2007).

Carbon fluxes in grassland ecosystems

An alternative to the direct measurement of C stock changes in grasslands is to measure the net balance of C fluxes (net C storage, *NCS*) exchanged at the system boundaries. This approach provides a high temporal resolution and changes in C stock can be detected within one year. In contrast, direct measurements of stock change require several years or several decades to detect significant effects, given the high variability among samples. The net carbon storage (*NCS*) is the arithmetic sum of the C fluxes crossing the boundary of the field investigated: i) trace gases exchanged with the atmosphere (*i.e.* CO_2, CH_4, volatile organic compounds, VOC, and emissions during fires), ii) organic C imports (manures) and exports (harvests, animal products), iii) dissolved C lost in waters (dissolved organic and inorganic C) and lateral transport of soil C through erosion (Eq. 1):

$NCS = (NEE - F_{CH4-C} - F_{VOC} - F_{fire}) + (F_{manure} - F_{harvest} - F_{animal-products}) - (F_{leach} + F_{erosion})$
(Eq. 1)

where *NEE* is the net ecosystem exchange of CO_2 between the ecosystem and the atmosphere, which is here conventionally positive for a C gain by the ecosystem. F_{CH4-C}, F_{VOC} and F_{fire} are trace gas C losses from the ecosystem (g C/m^2 per year). F_{manure}, $F_{harvest}$ and $F_{animal-products}$ are lateral organic C fluxes (g C/m^2 per year) which are either imported or exported from the system. F_{leach} and $F_{erosion}$ are organic (and/or inorganic C losses in g C/m^2 per year) through leaching and erosion, respectively.

Nevertheless, depending on the system studied and its management, some of these fluxes can be neglected for NCS calculation. For instance, fire emissions by grasslands are very low in temperate regions like Europe (*i.e.* below 1 g C/m^2 per year over 1997-2004), while they reach 10 and 100 g C/m^2 per year in Mediterranean and in tropical grasslands, respectively (Van der Werf *et al.*, 2006). Erosion ($F_{erosion}$) is also rather insignificant in permanent grasslands (*e.g.* in Europe), but can be increased by tillage in the case of sown grasslands. The global map of $F_{erosion}$ created by Van Oost *et al.* (2007) indicates that grassland C erosion rates are usually below 5 g C/m^2 per year, even in tropical dry grasslands (Van Oost *et al.,* 2008). The total dissolved C loss by leaching was estimated by Siemens (2003) and Janssens *et al.* (2003) at 11 ± 8 g C/m^2 per year for Europe. This flux tends to be highly variable depending on soil (pH, carbonate) and climate (rainfall, temperature) factors and it could reach higher values in wet tropical grasslands, especially on calcareous substrate. VOC emissions by grassland systems are increased in the short-term by cutting and tend to be higher with legumes than with grass species (Davison *et al.*, 2008). However, these C fluxes are usually small and can easily be neglected. Therefore, with temperate managed grasslands equation 1 can be simplified as (Allard *et al.*, 2007):

$NCS = (NEE - F_{CH4-C}) + (F_{import} - F_{export} - F_{animal-products}) - F_{leach}$
(Eq. 2)

With the advancement of micrometeorological studies of the ecosystem-scale exchange of CO_2 (Baldocchi and Meyers, 1998), eddy flux covariance measurement techniques have been applied to measure *NEE* in grasslands and wetlands. Ruminant's belched CO_2 (digestive + metabolic CO_2) emission at grazing (Pinares-Patino *et al.*, 2007) is a component of *NEE*. F_{CH4} is the sum of the CH_4-C flux exchanged (methane emission or oxidation) between the soil and the atmosphere and of the methane emission by the enteric fermentation of ruminants at grazing.

At one site, in an upland pasture in Central France, soil carbon stock change was directly measured after 4 and 8 yrs of continuous eddy flux covariance CO_2 measurements. Soil coring indicated, on average, a 35 % higher *NCS* than the eddy flux covariance estimate of *NCS* (Katja

Klumpp, unpublished). Therefore, direct soil measurements confirmed the occurence of soil carbon sequestration which was first determined from the eddy flux covariance measurements.

3. The net carbon balance of European grasslands

A network of grassland sites equipped for eddy flux covariance measurements of CO_2 exchanges with the atmosphere has been established in a range of national and EC funded research projects, starting with EC FP5 (Greengrass project, see Soussana *et al.*, 2007; Carbomont project), followed by FP6 (CarboEurope IP) and by the newly established ICOS infrastructure (Integrated Carbon Observation System). Through collaborations with these projects in the EC FP7 AnimalChange project, we have collected data from 39 sites spanning the European continent and including grasslands, wetlands and moorlands with a diversity of managements (grazing, cutting, abandoned, with or without inorganic and organic N fertilization). Each site has run, over at least two years, eddy flux covariance measurements of CO_2 exchanges with the atmosphere and measurements of organic C imports (manures) and exports (harvests, animal products). The annual carbon balance of these sites has been calculated according to Equation 2 and analyzed in order to determine the drivers of carbon sequestration across European grasslands.

These sites are from 15 European countries, spanning highly contrasting regions from the Arctic to the Mediterranean and from the Atlantic to central Europe. Each site has been measured between 1 and 11 years, resulting in a total of 213 site years.

Data include the site latitude and longitude, the vegetation type with three categories (permanent and sown grasslands, wetlands), the management type (grazing, cutting, mixed or abandoned), the annual N fertilizer supply (inorganic and organic), the annual means of air temperature and of precipitations and the annual Net Ecosystem Exchange (*NEE*) of CO_2. In addition, the lateral fluxes of organic carbon induced by herbage harvests and by manure supplies have been recorded at all sites. Moreover, with grazed sites carbon intake and carbon emissions to the atmosphere as methane from enteric fermentation have been estimated from the animal type, live weight and mean annual stocking density based on IPCC Tier 1 methodology. Finally, the exports of C in animal products (meat and milk) have been estimated as in Soussana *et al.* (2010c).

On average of the 213 site years data, the gross primary productivity (*GPP*, i.e. photosynthesis) reached 1218 ± 42.8 gC m^{-2} yr^{-1} (mean ± s.e.) with a mean net carbon storage (*NCS*) equal to 76 ± 11 gC m^{-2} yr^{-1} showing a significant carbon sequestration in European grasslands. According to a one-Sample Signed Rank Test , there is a statistically significant difference (P <0.001) between the NCS median and zero. The 95% range of the *NCS* median is 38 to 81 gC m^{-2} yr^{-1}(Tab 2). Approximately one fourth of the site years NCS had negative values, showing net carbon release by the grasslands. Kruskal-Wallis One Way Analysis of Variance on Ranks did not indicate a statistically significant difference in *NCS* across grassland types and between cut only and grazed only pastures.

Climate was a strong driver of *GPP*, with maximal *GPP* ($GPP_{max} = 2250$ gC m^{-2} yr^{-1}) reached at a mean annual temperature of 10.0 ± 0.5 °C and at a mean annual precipitation of 1240 ± 80 mm yr^{-1}. A Lorentzian model fitted to these climatic drivers explained 52% of the annual *GPP* variance across sites and years. There was no significant effect of management factors on annual net carbon storage, possibly because the management factors were confounded with climate factors. On average, net carbon sequestration reached $9.0 \pm 0.02\%$ of *GPP* in the absence of a lateral flux of organic carbon and of N fertilizer supply (i.e. unmanaged grassland). Consistent with a previous report (Soussana *et al.*, 2007), at a given *GPP* there was a highly significant (P<0.0001) increase in *NCS* with N fertilizer supply and with the net import of organic carbon (*Lc*, organic C balance between manure supply and exports by harvest and intake of digestible carbon, in kg C ha^{-1} yr^{-1}).

$NCS = (f_2 + k_N.Ns).GPP + k_C.Lc$, with: $Lc = Manure\text{-}Dig.Intake\text{-}Harvest$
(Eq. 3)

Where, Ns is the N fertilizer supply (kg N ha^{-1} yr^{-1}); f_2, k_N and k_C are numerical constants and Dig is the digestibility of the ingested DM. Hence, there is a clear trade-off between C sequestration and herbage use by cutting and grazing in grasslands. This trade-off is stronger at cutting since non-digestible carbon is returned to the soil during grazing. In unmanaged grasslands, Eq. 3 shows that NCS is a constant fraction ($f_2 = 0.09$) of GPP. In grazed only pastures, NCS is reduced by animal intake and is increased by manure supply. DM intake is a fraction of $ANPP$, the above-ground net primary productivity. This fraction (f_1) is the herbage use efficiency. In turn, $ANPP$ is a fraction f_0 (one third on average) of GPP. Hence:

$NCS = ((f_2 + k_N.Ns) /f_0.f_1).Intake + k_C.(Manure\text{-}Dig.Intake)$
(Eq. 4)

4. Can we create carbon neutral pastures in Europe?

For a grazed and unmanaged pasture developed on mineral soils, the IPCC Tier 1 method (IPCC, 2006) shows that emissions of CH_4 and N_2O are directly proportional to the intake of dry-matter by the grazing livestock. However, the Tier 1 method does not account for soil organic carbon stock changes (NCS) in pastures. The net GHG balance (kgCO$_2$ equivalents ha^{-1} yr^{-1}) of a pasture can therefore be corrected taking into account NCS as calculated by Eq. 2:

$GHG = Intake.(f_N. E_{N2O}.w_{N2O} + Dig. E_{CH4}. w_{CH4}) -NCS. M_{CO2}$
(Eq. 5)

Intake is the annual DM intake by the grazing livestock (kg DM ha^{-1} yr^{-1}), E_{N2O} and E_{CH4} are the N$_2$O and CH$_4$ emission factors at grazing calculated from default IPCC Tier 1 values. w_{N2O} and w_{CH4} are the warming potential of N$_2$O and CH$_4$ relative to CO$_2$ per unit weight on a 100 yrs time horizon (298 and 25, respectively, IPCC, 2006). M_{CO2} is the molar weight ratio of CO$_2$ to C (44/12). f_N is the fraction of N in herbage DM. Combining equations 3 and 4:

$GHG = Intake.(f_N.E_{N2O}.w_{N2O}+Dig.E_{CH4}.w_{CH4}-((f_2+k_N.Ns)/(f_0.f_1)-k_C.Dig).f_C.w_{CO2})+kc.Manure$
(Eq. 6)

When there is no manure applied, Eq. 6 simplifies and the herbage use efficiency ($f1^*$) for which GHG equals 0 (i.e. carbon neutral pasture) does not vary with intake and can be calculated as:

$f1^* = ((f_2+k_N.Ns)/f_0-k_C.Dig).f_C.w_{CO2} / (f_N.E_{N2O}.w_{N2O}+Dig.E_{CH4}.w_{CH4})$
(Eq.7)

For this critical herbage use efficiency ($f1^*$), intake can be calculated from GPP. Moreover, since GPP has been fitted to the mean annual temperature (T) and mean annual precipitation (P) using a Lorentzian model ($GPP = GPP_{max}/f(T).f(P)$), GPP can be estimated from climate conditions. Hence, assuming cattle grazing with a fixed DM intake per head (20 kg DM intake per day and per livestock unit, LSU), a critical stocking rate (SR^*, LSU ha^{-1}) leading to zero carbon emissions by a pasture can be calculated. These calculations for carbon neutral pastures were made considering three management options: i) no fertilization; ii) pastures supplied with mineral N fertilizers, ii) pastures supplied with manure (calculated from Eq. 6). The corresponding results are shown in Figure 1 for a range of T and P conditions covering the climatic conditions experienced by grasslands in Europe.

Without N fertilizer supply, the critical cattle stocking rate (SR^*) under optimal climate conditions can reach 0.85 LSU/ha over a grazing period of 200 days per year. With less favorable climate conditions, SR^* declines down to 0.3 LSU/ha (Fig. 1). However, these calculations assume a constant duration of the annual grazing season. Assuming, more

realistically, a reduced grazing duration in unfavorable climates implies that *SR** would not decline to the extent shown in Fig. 1a. A supply of one ton of fresh carbon as cattle manure with a C:N ratio of 14 leads to a rise in the critical stocking rate up to 1.2 LSU/ha (Fig. 1b), whereas the supply of mineral N fertilizer (100 kg N/ha) is relatively less efficient since *SR** does not exceed 0.93 LSU/ha (Fig. 1c). While manure supply has a large impact on *SR** in unfavorable climate (*SR** with manure is always above 0.6 LSU/ha), mineral N fertilizer has a small negative impact on *SR** in poor climates (minimum *SR** of 0.26 LSU/ha) (Figure 1).
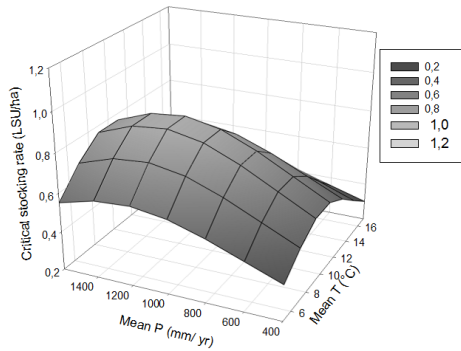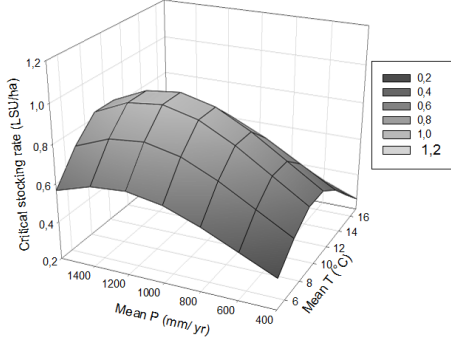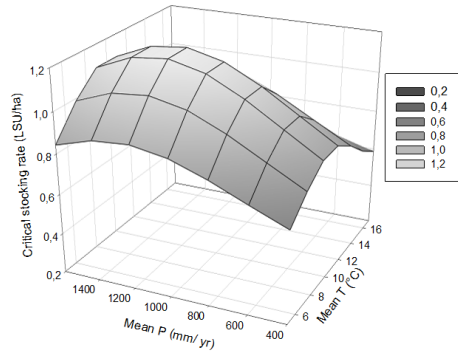
Figure 1. Critical stocking rate (in LSU, livestock units per ha during a 200 days grazing season) leading to a zero GHG balance of pastures which are unmanaged (a), fertilized with manure (supplying one ton C and 70 kg N/ha) (b) and supplied with inorganic N fertilizer (100 kg N/ha) (c).

These estimates of *SR** are consistent with previous results showing that grazing and cutting intensity reduces carbon storage in grasslands (Soussana *et al.*, 2007), which explains why *SR** values are usually low and are reached mostly under extensive grazing conditions. Indeed, herbage use efficiency (*f1,* the ratio of herbage intake to above-ground net primary productivity) has a low value when carbon neutrality is reached in pastures. With unmanaged pastures, *f1**=0.20 and this value is constant across the climatic space (Eq. 5). However, *f1** derivation in fertilized pastures requires numerical solving and values across climatic conditions vary with manure application between 0.29 and 0.40, and with N fertilizer application between 0.18 and 0.22.

These results show that carbon neutral pastures can be achieved at low livestock grazing density and confirm the concept of a critical livestock density proposed by Soussana and

Lemaire (2014) for environmentally sustainable grassland intensification. Further work will be required to apply this concept to realistic grazing systems by taking into account livestock needs for grassland roughage in winter (and in summer in the Mediterranean). Moreover, the above results are only valid on average. The exact SR^* value may vary across grassland fields depending on the soil type, on the vegetation cover, on the current soil organic carbon stock and on the abundance of N-fixing legumes. For instance, drained organic soils are unlikely to store C even under low grazing density conditions (Soussana *et al*., 2010).

The likely technical feasibility of carbon neutral pastures questions the possibility of voluntary carbon payments that could be offered to farmers managing 'carbon neutral pastures'. Such payments would aim at compensating for the loss of profitability created by the limitation in animal stocking density below SR^*. While this carbon mitigation option has a large technical potential, given the extent of grasslands in Europe, its cost would need to be assessed (see Pellerin *et al*., 2013) and compared to other options in the animal agriculture sector (e.g. biogas, changes in animal diets, *etc*.). This option could be combined with P fertilization in some depleted soils and with an increased use of pasture legumes which would further reduce N_2O and CH_4 emissions (Luescher *et al*., 2014). Silvo-pastoral systems which are currently being developed in the wet tropics could also be increasingly used to strengthen the grassland carbon sink in the temperate zone (Pellerin *et al*., 2013).

5. The climate sensitivity of grassland carbon stocks

Grassland production is intimately linked to climate conditions and therefore highly exposed to climatic variability and climate change. Between 1980 and 1999, severe droughts have caused mortality rates in national herds of between 20% and 60% in several arid sub-Saharan countries (IPCC, 2007). The extreme drought and heatwave that hit Europe in the summer of 2003 was unprecedented since at least 1500. It caused a green fodder deficit of up to 60% in affected countries like France. In Switzerland fodder had to be imported from as far away as Ukraine. In Australia, the widespread six-year drought from 2001 to 2007 is considered the most severe in the nation's history and had large negative impacts on livestock production.

A further drying of large parts of the subtropics is likely by the end of this century (IPCC, 2013). For instance, in Europe, in the next 40 years, the risk of summers as warm as 2003 may increase by two orders of magnitude and may approach the norm by 2080 under high emission scenarios. Increased aridity and persistent droughts are projected in the twenty-first century for most of Africa, southern Europe and the Middle East, most of the Americas, Australia and South East Asia (Field *et al*., 2012). A number of these regions have a large fraction of their land use covered by grasslands and rangelands. Projected increases in climate variability and increases in the length of the dry summer period is likely to impact negatively on ground cover in Mediterranean climates and in drylands, increasing soil erosion risks (Crimp *et al*., 2010).

A probabilistic risk analysis can be developed, by defining risk as the product of hazard probability (e.g. the probability of drought occurrence) and the response to hazard (Van Oijen *et al*., 2013). With this approach, a significant increase in exposure to summer drought risk was evidenced for French grasslands (Graux *et al*., 2013). Simulated future conditions show an increased inter-annual and seasonal variability of grassland production. Dairy production at grazing in summer is estimated to drop down below one-third of the current median value in four out of 30 years for 2070–2099, whereas similar shortfalls were not observed with the baseline climate (Graux *et al*., 2013). A detailed analysis of risks under the A1B emission scenario further shows that European grasslands could shift from a carbon sink to a carbon source for the atmosphere in extreme years (Van Oijen *et al*., 2014).

The Agricultural Model Intercomparison and Improvement Project (AgMIP) has initiated a Coordinated Climate-Crop Modeling Project (C3MP) with a protocol first established for arable crops (Ruane *et al*., 2014). This protocol has been adapted to grassland systems

considering specific management recommendation, with first focus on temperate grasslands. The impact of climate change on future greenhouse gas emissions and removals from grassland systems is explored by utilizing site-calibrated models to provide projections under probabilistic climate change scenarios. These scenarios are defined by a combination of air temperature, precipitation and CO_2 atmospheric rate changes.

This protocol has been applied to a temperate grassland site, assuming a 200 days grazing season with mineral N fertilizer supply and a herbage use efficiency of 0.2 (close to f_1* under current climate conditions). Results show that a rise in air temperature and in precipitation departs the pasture from carbon neutrality and leads to a large rise in GHG emissions per unit area (Fig. 2a). Moreover, as the pasture productivity declines with warming and reduced precipitation the GHG balance per head increases exponentially (Fig. 2b). Therefore, climatic variability has a strong potential to shift the carbon sink of grasslands to a carbon source. These results do not account, however, for the long term acclimation of grasslands to climate change through changes in vegetation which may increase pasture resilience.
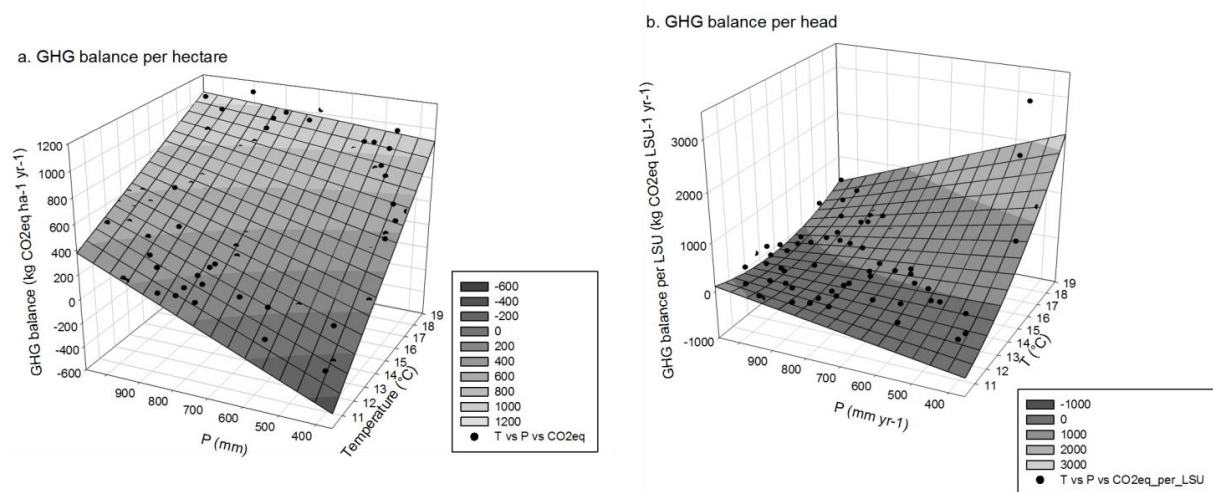
Figure. 2. Simulated GHG balance of a temperate grassland for a range of temperature and precipitation conditions (following the C3MP protocol, see Ruane *et al*., 2014). GHG balance was expressed per unit pasture area (a) and per head (b) assuming a 200 days grazing season with one cattle livestock unit. A herbage use efficiency value of 0.2 (close to *f1**, see text, under current climate conditions) was assumed.

Conclusion

The carbon sink of European grasslands results from past changes in management (grassland fertilization, reduction in stocking density) and from global change (rise in atmospheric CO_2 and warming). This carbon sink can be managed by grazing and by fertilization. A better understanding of the role of these drivers and of their interactions with soil and vegetation types may allow designing guidelines for carbon neutral pastures. Such carbon neutral pastures are extensive and extensification is currently not economically viable in the absence of funding from carbon markets or targeted agricultural subsidies. Priority should therefore be given to the restoration of degraded pastures which offers win-win possibilities by combining increases in plant productivity, in soil carbon stocks and in animal production. Such a scheme has already been successfully introduced in Portugal (see www.terraprima.pt/pt/) using phosphorus fertilization and species rich grass-legume mixtures. However, soil organic carbon stocks are vulnerable to changes in agricultural management and to climate change. Therefore, the mitigation of CH_4 and N_2O emissions from animal production is required at all stages of livestock supply chains in addition to efforts aiming at pasture restoration and transition towards carbon neutral pastures.

Acknowledgements

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Theme 1 submitted papers

Reducing greenhouse gas emissions in silage production with oxygen barrier film

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Abstract

Farm-scale bunker silos were filled with whole-crop maize (*Zea mays* L.) and their top surfaces covered with either two layers of standard low-density polyethylene film of 150μm thickness, following normal practice, or a single layer of low-density oxygen barrier (OB) film of 45μm thickness. Total weight of film used per silo was 241.5 kg for standard film and 43.4 kg for OB film. Primary energy used per silo for the manufacture of the films was 18.9 GJ for standard film and 3.39 GJ for OB film. Estimated global warming potential of film used per silo was 514.4 kg CO_2e for standard film and 92.3 kg CO_2e for OB film. Mean composition of samples of silage taken from the top 30 cm of each silo was similar between the two types of film. The use of OB film reduced primary energy use and greenhouse gas emissions associated with film by 82% without affecting silage composition adversely.

Keywords: silage, polyethylene, greenhouse gases

Introduction

A bunker silo of 40 m length, 12 m width and 2.5 m height contains almost 20% of the original ensiled crop in the top 0.5 m. Studies with 127 farm-scale silos in the USA over a four-year period revealed that loss of organic matter (OM) during the storage period was 470 g kg^{-1} in the top 0.5 m of uncovered silos, compared to 113 g kg^{-1} for the same silage 0.5 m to 1 m from the top surface (Bolsen, 1997). These losses illustrate the importance of maintaining an effective barrier to both water and oxygen throughout the storage period. Covering silos with polyethylene film reduces losses by protecting the crop from the effects of wind and rain and also by reducing, but not preventing, oxygen permeation into the silo. Normal practice in northern Europe is to line the side walls with a single sheet of film that overlaps the periphery of the top surface, and to use two layers of film to cover the top surface itself.

Global consumption of low-density polyethylene film for silage was 582.5 kt in 2012 (Wordpress, 2013). We estimate that 368 kt of polyethylene film are used annually worldwide to cover walled bunker and unwalled clamp silos and 156 kt of stretch-film to wrap baled silage. The production of low-density polyethylene is associated with the consumption of 78.1 MJ primary energy kg^{-1} and with a global warming potential (GWP) of 2.13 kg CO_2e kg^{-1} (Plastics Europe, 2008). Additional energy is used in the recovery and recycling of film and initiatives have been launched in some member states of the European Union to encourage the recycling of agricultural plastics. For example, in France farmers are charged €65 t^{-1} of film to support a recycling programme (Comité Français des Plastiques en Agriculture, 2012).

Losses of nutrients to the atmosphere through the aerobic deterioration of silage increase greenhouse gas (GHG) emissions per unit of animal product output and have a large negative impact on net return to labour and management (Van Schooten and Phillipsen, 2012). The use of oxygen barrier (OB) film to cover silos and bales is associated with reduced losses of organic matter during the storage period and increased aerobic stability of silage in the peripheral areas of silos, compared to standard polyethylene film (Wilkinson and Fenlon, 2013). In this paper

the hypothesis was tested on farm-scale silos that the use of OB film reduced GHG emissions in silage production compared to standard polyethylene film.

Materials and methods

Two adjacent walled bunker silos of 40 m length and 12 m width at the Waiboerhoeve Research Farm of Wageningen UR Livestock Research, Lelystad, The Netherlands, were filled with chopped whole-crop forage maize (*Zea mays* L.) between 15 and 20 October 2012. Both silos were filled to an average height of 2.2 m. Harvesting and ensiling equipment were identical for both silos and about 700 t of fresh crop were ensiled in each silo. The top surface of one silo was covered immediately with two sheets of standard low-density polyethylene film (RKW ProAgri®, Michelstadt, Germany), following normal practice on the farm. Each sheet was of 50 m length, 14 m width, 150 μm thickness and 0.92 g cm^{-3} density. A third sheet of the same standard polyethylene film (50 m length x 7 m width x 150 μm thickness, 0.92 g cm^{-3} density) was used to line the side walls. The top surface of the other silo was covered immediately with a single sheet of OB film comprising low density polyethylene co-extruded with ethylene vinyl alcohol (Supastop®, B Rimini Ltd, London, UK) of 50 m length, 14 m width, 45 μm thickness and 0.93 g cm^{-3} density. A second sheet of the same OB film (50 m length and 5 m width, 60 μm thickness and 0.93 g cm^{-3} density) was used to line the side walls. Woven polypropylene netting (Genap BV, 's-Heerenberg, The Netherlands), weighed down by gravel bags, was placed above the top sheets of both silos. Six samples of silage of 1 kg fresh weight were taken for compositional analysis (BLGG AgroXpertus, Wageningen, The Netherlands) to 30 cm depth from the top surface and 2 m from the outer walls from each silo during the feed-out periods; on 26 June 2013 (251 days post-ensiling) for the silo covered with OB film and on 11 September 2013 (328 days post-ensiling) for the silo covered with standard film.

Results

Primary energy use and GHG emissions, estimated as GWP, associated with the two films are shown in Table 1. The use of OB film reduced total weight of film, primary energy use and GWP from film by 82% compared with standard film. Additional benefits of lower mass of film to be recycled, with further reductions in GHG, would also accrue.

Table 1. Weight of film used per silo, associated primary energy use and global warming potential (GWP): Standard film compared to OB film

	Standard film	OB film
Total weight of film (kg per silo)	241.5	43.4
Primary energy @ 78.1 MJ kg^{-1} film (GJ)	18.9	3.39
GWP @ 2.13 kg CO$_2$e kg^{-1} film (kg)	514.4	92.3

The mean composition of the silages in the top 30cm stored under standard polyethylene or OB film is shown in Table 2. Differences in mean composition between silages stored under standard and OB film were relatively small. With the exception of water soluble carbohydrates, lactic acid and acetic acid, coefficients of variation for compositional parameters were less than 10% for silages stored under both types of film.

Table 2. Composition of silages (mean ± SD of six samples) in the top 30cm stored under either standard polyethylene or oxygen barrier film

	Standard film	OB film
Dry matter (DM, g kg^{-1})	342.0 ± 1.7	335.7 ± 21.3
Composition (g kg^{-1}DM)		
Ash	35.5 ± 2.7	41.3 ± 4.1
pH	4.1 ± 0.1	4.1 ± 0.2
Crude protein	64.0 ± 1.9	66.8 ± 1.3
Water soluble carbohydrates	13.3 ± 4.8	15.5 ± 5.0
Starch	400.8 ± 26.9	382.2 ± 31.2
Neutral detergent fibre	369.2 ± 34.0	367.0 ± 18.2
Acid detergent fibre	201.2 ± 19.3	205.3 ± 14.0
Digestible organic matter	726.0 ± 22.1	716.5 ± 14.6
Lactic acid	42.0 ± 5.1	31.8 ± 11.8
Acetic acid	13.2 ± 4.7	17.3 ± 2.1

Conclusions

Covering ensiled forage maize with a single layer of OB film gave large reductions in primary energy and GHG associated with polyethylene film, compared with normal practice of covering with two layers of standard film. The composition of silage in the top 30 cm was similar between the two types of film.

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Effects of fertilization and soil compaction on nitrous oxide (N2O) emissions in grassland

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Abstract

The effect of fertilization and soil compaction on nitrous oxide (N_2O) emissions in pure grass and mixed grass-clover leys was assessed at Fureneset in western Norway. The experiment was divided into two measurement periods. The first period lasted from 30 April to 29 May, following the application of cattle slurry supplying 110 kg total N ha^{-1}. The second period lasted from 3 to 30 July, following 60 kg total N ha^{-1} as mineral NPK applied after the first cut. Cattle slurry did not lead to a transient increase in N_2O emissions, indicating a low mineralization rate at low temperatures in spring 2013. Soil compaction increased N_2O emissions only in the mixed grass-clover ley. The application of mineral fertilizer after the first cut induced transiently high N_2O emissions, which tended to be higher in compacted than non-compacted plots. The N_2O emissions from mixed grass-clover ley were negligible during the second experimental period, indicating that clover can substitute for the input of mineral fertilizer and thus mitigate N_2O emissions during the growing season.

Keywords: cattle slurry, clover, gaseous emissions, grasses, mineral fertilizer, nitrogen.

Introduction

Agriculture is responsible for a large part of the atmospheric loading of nitrous oxide (N_2O). The main drivers of N_2O emissions are the soil microbial processes of nitrification and denitrification. Several studies indicate that N_2O emission rates depend on soil physical environment and management practices. Soil compaction is a common form of soil structure degradation. Due to reduction of total soil porosity and changes in pore size distribution, the mineralization rate of nitrogen (N) and carbon can be reduced (Breland and Hansen, 1996) and air permeability and gas diffusivity altered (Ball *et al*., 2008). These factors may increase anaerobic microsites in soil that may lead to higher N_2O emissions. The management of productive grasslands typically involves cutting and/or grazing, and fertilizer application. The addition of N-fertilizers not only stimulates growth of plants, but also increases the potential of direct N_2O losses. The objective of this study was to determine the effect of fertilization and soil compaction on gaseous N_2O emissions in pure grass and mixed grass-clover leys.

Materials and methods

The N_2O flux measurements were carried out on third-year leys on sandy loam with pH 5.9 at Fureneset (61° 22' N 5° 24' E) in western Norway. The field trials were harvested twice a year. Two levels of two wheel-by-wheel passes with tractor traffic were introduced after each harvest: no traffic/ no soil compaction (NC) and traffic with heavy tractor (7 t)/compaction (C). Pure grass and grass-clover leys were included in the experiment. The dominating grass species were timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* L.) with a visually determined content of approximately 80-95% of dry matter yield in the pure grass ley. In the mixed ley the content of red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens*

L.) together was 20-30% at first cut and 40-60% at second cut. The content of grasses was reduced correspondingly.

The experiment was divided into two measurement periods. The first N_2O measurement period lasted from 30 April to 29 May. The first gas sampling occurred shortly before cattle slurry, supplying 110 kg total N ha^{-1}, was applied to all experimental plots. Then gas samples were collected after two hours, and then at 1, 4, 6, 8, 13, 20 and 27 days after application. The second N_2O measurement period lasted from 3 to 30 July. Half of the pure grass plots received 170 kg total N ha^{-1}, of which 60 kg N ha^{-1} was applied after the first cut as mineral fertilizer. Similar to the previous measurement period, the first gas measurement took place shortly before fertilization and then gas samples were collected 1, 2, 5, 7, 9, 13, 16 and 27 days after application. N_2O flux was measured using static chambers. One aluminium frame (52 cm x 52 cm x 25 cm) per plot was inserted to a depth of 10-12 cm shortly before the first N_2O measurement and left in the soil throughout the measurement period. The frames had a groove filled with water to ensure air-tight connection with 20-cm high vented aluminium chamber. Air samples were taken from the chamber headspace at regular intervals (0, 15, 30, 45 min), using a 20-mL air-tight polypropylene syringe. The samples were injected into pre-evacuated 12-mL glass vials and analysed by a gas chromatograph.

Data of actual air and soil temperature and precipitation during the experimental periods were taken from local weather station at Fureneset (Figure 1B and D).

The experiment was a completely randomized split plot block design with three replicates. Analysis of variance (General Linear Model) was used according to a split-split plot model to evaluate the significance of tractor traffic (main plots), fertilization type/level (split plots) and seed mixture (split-split plots) on cumulative N_2O emissions.

Results and discussion

The N_2O fluxes, generally, were low after the cattle slurry had been applied in spring (Figure 1A). Surprisingly, no short-term N_2O emission peaks were measured after the application. This is in contrast with previous studies under the same climate conditions (Rivedal *et al.*, 2013). Relatively low soil temperatures at the beginning of the experiment (Figure 1B) probably resulted in low mineralization rates and low gaseous losses from applied cattle slurry. One day after the application, however, N_2O emissions in the compacted grass-clover stands were significantly greater than in compacted and non-compacted pure-grass stands and in non-compacted grass-clover mixtures (Figure 1A). This pattern remained over the entire one-month experiment. These results suggest that more N was available for microbial transformation in grass-clover stands than in pure-grass stands in spring.

As expected, the application of mineral fertilizer after the first cut induced transiently high N_2O emissions (Figure 1C). Emissions were highest on the second day after the application and decreased significantly thereafter. Difference between fertilized and unfertilized treatments remained for ten days. The effect of soil compaction after the first cut on the N_2O production was less, as expected. There was a significant interaction between the soil compaction and fertilizer application on the first day after fertilization. Limited precipitation before tractor traffic resulted in dry soils and may have limited the effect of altered soil structure. It has been demonstrated that more N_2O is produced in response to soil drying and rewetting than to soil compaction (Beare *et al.*, 2009). This experiment clearly showed that the presence of clover in the sward did not affect N_2O production during the growing season (Figure 1C). This is in accordance with a recent study in Norway which found a statistically measurable impact of clover on N_2O emissions only in a dry and warm year but not in a wet and cold year (Hansen *et al.*, 2014). Thus, use of clover in leys may minimize direct N_2O losses from synthetic fertilizers, depending on the annual weather conditions.
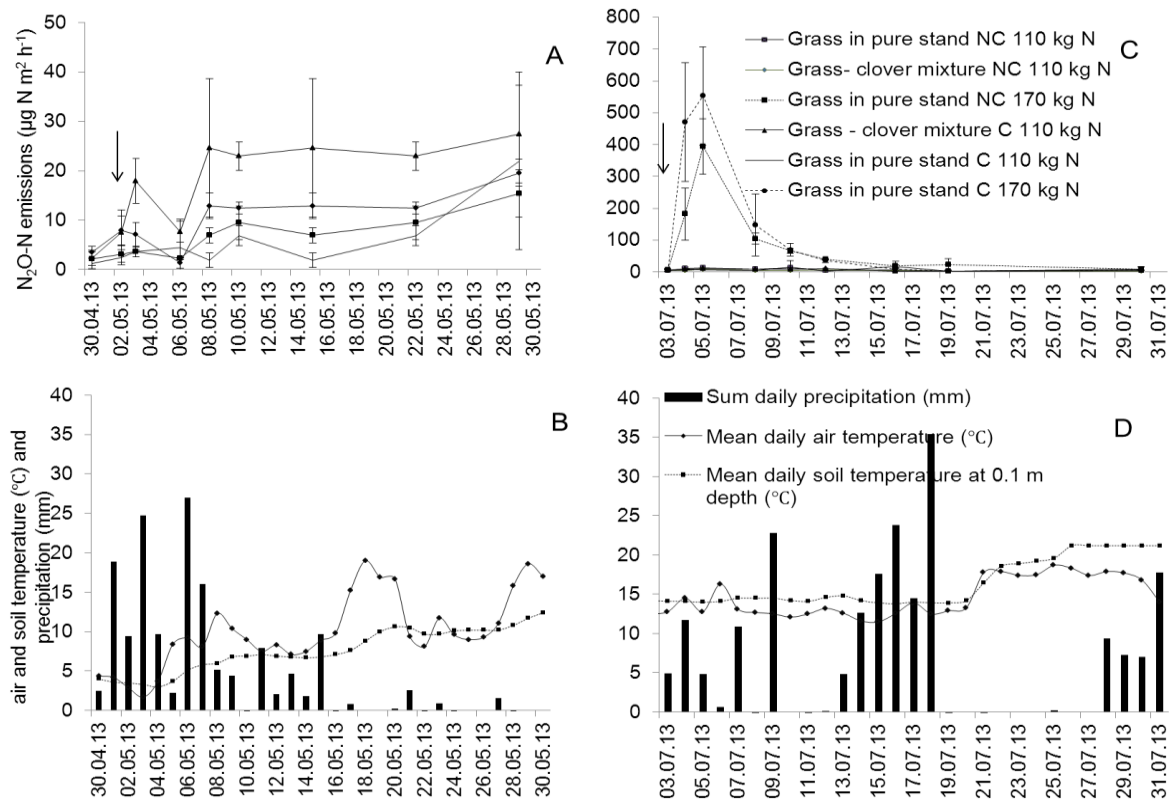
Figure 1. N_2O emissions from the non-compacted (NC) and compacted (C) pure grass and grass-clover mixture after application of 110 kg total N/ha as cattle slurry on 2 May (1A) and after application of 60 kg total N/ha as mineral N on 3 July (1C). Figures 1B and 1D show mean daily air and the soil temperature and sum of daily precipitation. Arrows denote the time of N application.

Conclusion

Cattle slurry applied in spring did not increase N_2O emissions in pure grass and grass-clover stands in a cold year. However, the presence of clover had some impact on N_2O flux, particularly in plots subjected to heavy tractor traffic the year before. Use of mineral fertilizer to pure grass after the first cut enhanced N_2O emissions immediately after the application, and high fluxes lasted for approximately one week. N_2O emissions from mixed grass-clover swards were negligible. Thus, inclusion of clover in a sward may minimize the risk of direct environmental pollution during the growing season.

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Modelling livestock and grassland systems under climate change

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Abstract

The livestock and grassland theme (LiveM) of the MACSUR (Modelling Agriculture with Climate Change for Food Security) (www.macsur.eu) knowledge hub brings together partners from across Europe to develop a pan-European modelling capability in the area of livestock systems modelling of climate change adaptation and mitigation. Through the project, inventories of grassland, animal and farm-scale models, as well as datasets related to grasslands and livestock have been compiled. Model inter-comparisons have taken place for grassland models, and a model evaluation protocol is being developed. Farm-scale modellers are undertaking a model inter-comparison exercise, and the theme has formed links to related projects in order to bring together a more coherent livestock systems modelling community. The need for better knowledge exchange within the livestock research community has been highlighted within the project, and is a focus for further action. The knowledge hub concept creates an arena for collaboration between research groups, disciplines and projects essential for tackling complex global issues such as the impact of climate change on agriculture.

Keywords: climate change, grasslands, integration, livestock systems, models

Introduction

The 'knowledge hub' concept within FACCE-JPI (Agriculture, Food Security and Climate Change Joint Programming Initiative) (www.faccejpi.com) is focussed on facilitating the creation of collaborative, inter-disciplinary structures for research, developing the coherence of purpose required to efficiently tackle urgent global and multi-sectoral problems such as climate change (Holzinger *et al.*, 2012; Soussana *et al.*, 2012). The MACSUR knowledge hub brings together 74 organizations from 17 European countries and Israel. It has the aim of developing a pan-European agricultural modelling capability, bringing together modelling teams within and between disciplines to improve the accuracy of predictions of the effects of climate change adaptation and mitigation on European agricultural systems. The project connects crop, trade, livestock and grassland modellers, and is focussed on collating, sharing and evaluating datasets for modelling use, developing methods of model inter-comparison, exploring ways to improve the impact and relevance of modelling outputs, and scaling up model predictions to the regional level.

A previous scoping paper detailed the priorities and opportunities provided by MACSUR for crop modelling (Rötter *et al.*, 2013). The paper presented here focuses on the LiveM theme, which deals with modelling livestock systems, including grasslands. Modelling such systems is complex, requiring the input of both physiological and management data, including for example the choice and nutritional constituents of feeds. An important challenge for LiveM is to develop awareness of the value of modelling to the wider livestock and grassland research community; for example, models can be used to demonstrate the potential real-world impacts

of experimental findings, aiding the successful communication of complex issues to stakeholders and policy-makers. Given the disparate nature of the livestock research community, the first priority for LiveM has been to develop links between research groups and between projects, facilitating the development of a mutual understanding of approaches and research needs.

Materials and methods

In order to achieve the aims of the LiveM theme within MACSUR, four work-packages (WPs) tackle different aspects of developing integrated modelling capabilities:

WP1: Collation and exploration of datasets on animal disease, dairy cow bio-meteorology and C sequestration in grasslands. Production of an inventory of animal-scale livestock models, and the development of online databases to share information.
WP2: Identifying grassland models and datasets, developing methods of data evaluation and model inter-comparison and creating a protocol for model evaluation.
WP3: Identifying farm-scale models and creating an online inventory, undertaking model inter-comparisons to assess the state-of-the-art in farm-scale modelling, and assessing the impact of mitigation policies on livestock systems.
WP4: Examining methods of scaling up livestock models in order to investigate the regional impacts of climate change, including methods for stakeholder involvement.
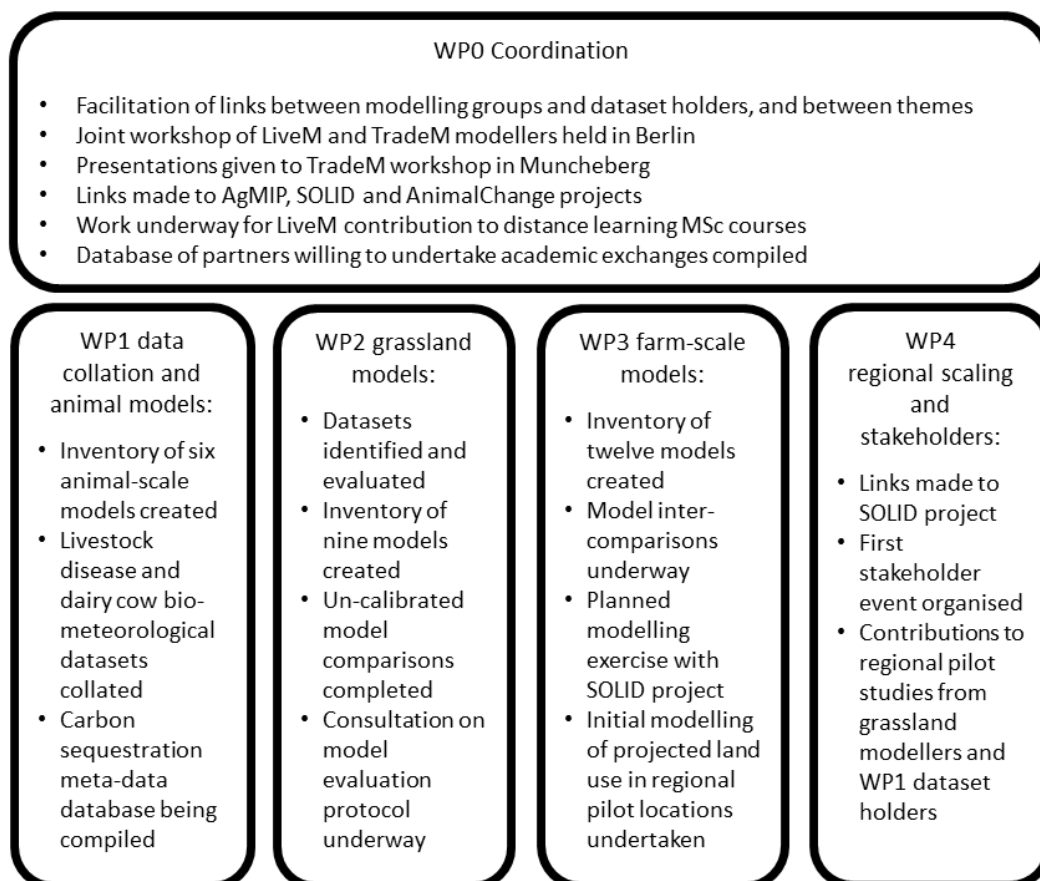
Figure 1. Overview of progress in LiveM. Note: acronyms: SOLID - Sustainable, Organic and Low Input Dairying project, AgMIP - Agricultural Model Intercomparison and Improvement Programme (Rosenzweig *et al.*, 2012), TradeM – trade modelling theme.

For all WPs, a central focus will be to contribute to cross-theme regional pilot studies, which bring together the methods and collaborations developed within each theme to model climate change scenarios in northern, central and southern Europe.

Results and discussion

Within LiveM, good progress has been made across all work-packages (Figure 1).
Bellocchi *et al.* (2013a, b) provide more detail on the achievements of grassland modellers in WP2. Work to develop links between modellers and dataset holders has proved a challenge, so that the creation of online inventories of meta-data has emerged as a priority in the formation of a more cohesive research community. The development of links with related projects is an essential step in building the capacity to address complex global issues in a joined-up way, using the pooled resources of different research groups, projects and disciplines. Providing the space for such interactions is a key advantage of the knowledge hub set-up, which creates an arena of exchange from which future integrated research is generated.

Conclusion

There is an urgent need for livestock and grassland researchers to develop a more coherent approach to the complex challenges facing the sector, through the development of resources which facilitate increased understanding between groups and disciplines. The MACSUR knowledge hub is an important step in developing a joined-up approach to livestock research. The ultimate aim must be to realise the potential of a more integrated research community that effectively links experimental researchers, modellers, stakeholders and policy-makers.

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Multiple regression analysis of the relationship between bioclimatic variables and grazing season length on European dairy, beef and sheep farms

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Abstract

The ability of bioclimatic variables to predict the grazing-season length on European dairy, beef and sheep farms was tested using stepwise multiple regression. Nineteen bioclimatic variables were sourced from the BIOCLIM project and grazing-season length data were sourced from the 2010 EUROSTAT Survey on Agricultural Production Methods. The experimental units were 987 European NUTS regions (nomenclature of territorial units for statistics) of which 703, 774 and 857 had grazing season length recorded for dairy, beef and sheep farms respectively. Bioclimatic variables accounted for an R^2 of approximately 0.60 for all three farm types, although the variables selected differed between sheep farms and dairy/beef farms. However, for all three farm types, cold weather limitations had the greatest effect on grazing season length, with the mean temperature of the coldest quarter resulting in R^2 values of 0.55 and 0.53 on dairy and beef farms, respectively, and the minimum temperature in the coldest quarter resulting in an R^2 of 0.52 for sheep farms. These results will enable some estimations of potential impacts of climate change on grazing management in Europe, although other sources of variation may need to be addressed first.

Keywords: grazing, season, climate, dairy, beef, sheep

Introduction

Grazing-season length is an important component of many ruminant production systems. It can influence production cost, environmental impacts, animal welfare and livestock disease transmission. The BIOCLIM bioclimatic variables are biologically meaningful climate variables on annual trends, seasonality and extreme (potentially limiting) climatic factors that are widely used to predict ecological niches and species geographic distributions (Booth *et al.*, 2014). These bioclimatic variables may also influence the management of livestock on commercial farms. For example, grazing-season length on farms may be influenced by these bioclimatic variables through their impact on grass growth, animal welfare and land trafficability. The objective of this study was to test the ability of bioclimatic variables to predict grazing season length in European regions using multiple regression analyses.

Materials and methods

Grazing season length data were sourced from the 2010 EUROSTAT Survey on Agricultural Production Methods (SAPM). Full SAPM methodological details are at: http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Glossary:Survey_on_agricultural_production_methods_(SAPM). Grazing-season length was defined as the number of months when livestock had at least some daily access to pasture. Weighted mean grazing season lengths for dairy, beef and sheep farm enterprises (standard EU farm typology: EU Commission Regulation No 1242/2008) were calculated for NUTS (nomenclature of territorial units for statistics) regions in 33 countries across Europe (NUTS 2 in Germany, NUTS 3 in the other 32 countries). There were 978 NUTS regions in total, of which 703, 774 and 857 had grazing-season length recorded on dairy, beef and sheep farms, respectively. Zero-grazing farms were excluded in order to focus only on farms that practised grazing. Gridded (1 km)

datasets on bioclimatic variables were downloaded from the WORLDCLIM website (www.worldclim.org) and processed using QGIS® to give regional means for each of the NUTS regions described above. There were 19 bioclimatic variables available (Table 1). The relationships between grazing-season lengths and these variables were then analysed using a stepwise multiple linear regression model with the GLMSELECT procedure in SAS®.

Table 1. WORLDCLIM (www.worldclim.org) bioclimatic variables based on temperature (temp: °C) and precipitation (prec: mm) records between the years 1950 and 2000.

BIO1:	Annual mean temp	BIO11	Mean temp of coldest quarter
BIO2:	Mean diurnal temp range	BIO12	Annual prec
BIO3:	Isothermality (Bio2 ÷ Bio7)	BIO13	Prec of wettest month
BIO4:	Temp seasonality (standard deviation)	BIO14	Prec of driest month
BIO5:	Max temp of warmest month	BIO15	Prec seasonality (coefficient of variation)
BIO6:	Min temp of coldest month	BIO16	Prec of wettest quarter
BIO7:	Temp annual range (Bio5 - Bio6)	BIO17	Prec of driest quarter
BIO8:	Mean temp of wettest quarter	BIO18	Prec of warmest quarter
BIO9:	Mean temp of driest quarter	BIO19	Prec of coldest quarter
BIO10:	Mean temp of warmest quarter		

Results and discussion

Bioclimatic variables were significantly associated with grazing season length for all three farm types and it was primarily cold temperature limitations that had the largest effect on grazing season length (Table 2). For dairy and beef farms, BIO11 accounted for the majority of the final model R^2 and BIO6 accounted for the majority of the R^2 on sheep farms. Low temperatures can reduce grass growth and result in animals being housed for their welfare (Hahn, 1981) and could therefore restrict grazing season length. The difference in variables selected on sheep farms (BIO6) as opposed to dairy/beef farms (BIO11) may be due to the generally longer grazing season on sheep farms (as shown in the dependant mean in Table 2) and therefore the greater likelihood that more extreme measures of cold temperature would become the limiting factor. However, it should also be noted that BIO11 and BIO6 were very closely correlated with each other ($R^2 = 0.98$) and that excluding BIO6 from the model resulted in it being replaced by BIO11 for sheep farms, with little change to the final R^2 (0.615 to 0.610) or root mean square error (1.227 to 1.228) and no change to the final selection of other variables.

The other bioclimatic variables that were selected by the model had a much smaller effect on the R^2 values (Table 2). The biological significance of these variables for grazing season length are more difficult to interpret and, in some cases, may be questionable. Isothermality (BIO3) is a quantification of how large the diurnal temperature range is in comparison to the annual temperature range and it was generally highest in the south-western regions of Europe. However, there are no obvious biological reasons why it should increase grazing season length. In contrast, BIO15 reduced the grazing season on sheep farms, possibly because declining summer precipitation along with increasing winter precipitation could reduce grass growth rates and land trafficability. However, why this was significant for sheep farms and not dairy or beef farms remains unclear. The negative effect of BIO18 (Table 2) on grazing-season length is also surprising. BIO18 can be generally be interpreted as summer rainfall, which is unlikely to be a limiting factor for grazing-season length in most regions of Europe. However, BIO18 is greatest around the Alpine, Carpathian and Kjolen mountain ranges where grazing-season length may be limited by other factors such as cold temperatures and soil characteristics.

It should be noted that grazing-season length as recorded by the SAPM does not include any measurements of daily access time to pasture or feed supplementation while at pasture. Therefore, grazing-season length does not directly reflect the importance of grazing for

milk/meat production on each farm because of feed supplementation within the grazing season. Furthermore, other factors such as land availability and land quality could also influence grazing-season length.

Table 2. Multiple regression analysis of the effects of bioclimatic variables on regional grazing season length in Europe. Only variables with a significant effect ($P < 0.05$) were included in the final model (stepwise selection).

	Dairy farms		Beef farms		Sheep farms	
	Effect (SE[a])	R^{2b}	Effect (SE[a])	R^{2b}	Effect (SE[a])	R^{2b}
Intercept	3.06 (0.454)		4.72 (0.439)		7.10 (0.491)	
Coefficients:						
BIO11 (mean temp of coldest quarter)	0.15 (0.019)	0.55	0.13 (0.019)	0.53		
BIO6 (min temp of coldest month)					0.16 (0.019)	0.52
BIO15 (prec seasonality)					-0.032 (0.003)	0.56
BIO3 (isothermality)	14.7 (1.526)	0.58	12.81 (1.468)	0.56	14.56 (1.714)	0.59
BIO18 (prec of warmest quarter)	-0.005 (0.0007)	0.61[c]	-0.007 (0.0007)	0.60[c]	-0.006 (0.0007)	0.62[c]
Number of regions	703		774		857	
Dependant mean (months)	6.81		7.66		8.27	
Model P value	<.001		<.001		<.001	
Root MSE[d]	1.15		1.21		1.23	

[a]Standard Error. [b]Progression of model R^2 as each variable was included. [c]Final R^2 of the model.

Conclusion

A number of bioclimatic variables predicted grazing-season length and could account for up to approximately 0.60 of the variation across farm types. In particular, cold temperature limitations (BIO6 and BIO11) had the greatest effect and appeared to be the most biologically meaningful. These results may enable some estimations of the impact of climate change on grazing-season length in Europe. However, approximately 0.40 of the variation in grazing-season length on European dairy, beef and sheep farms was not explained by bioclimatic variables and the inclusion of non-climatic factors may therefore be required.

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Performance of legumes for potential use in pasture swards under conditions of periodic water limitation

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Abstract

In Central Europe, the yield stability of grass-*Trifolium repens* mixtures may be at risk in the face of climate projections predicting periods of reduced rainfall or drought in summer, because yields of *T. repens* strongly decrease when water supply is limited. So far, there is little knowledge of the agronomic potential under drought conditions of alternatives to *T. repens*. In the present study we examined dry matter yield and water-use efficiency of five legume species that are currently of minor importance in pasture swards. Our data showed high dry matter yield stability and water-use efficiency for *Medicago lupulina* and indicate that this species warrants further consideration as an alternative to *T. repens* in pasture swards under conditions of limited summer precipitation.

Keywords: Dry matter yield, agronomic water-use efficiency, intrinsic water-use efficiency, stable carbon isotope

Introduction

Climate projections for Central Europe predict periods of reduced rainfall or drought in summer. In temperate pastures, this may set the yield stability of grass-white clover (*Trifolium repens*) mixtures at risk due to the high water requirement of white clover (Rochon *et al.*, 2004). Besides advances in breeding for white clover cultivars that feature an improved drought tolerance, the use of other legume species for pasture sward mixtures may offer an alternative. However, knowledge about their agronomic potential under drought conditions is limited. In the present study, we examine dry matter yield, water consumption and water-use efficiency of five legume species that are currently of minor importance in pasture swards.

Material and methods

The study was carried out from 2009 to 2011 at Göttingen, Germany, as a two-factorial (plant species; level of water supply: limited vs. well-watered control) container experiment in an unheated greenhouse. We used *Lotus corniculatus* L. ('Bull'), *Lotus uliginosus* Schkuhr (wild seeds), *Medicago lupulina* L. ('Ekola'), *Medicago falcata* L. (wild seeds), and *Onobrychis viciifolia* Scop. ('Matra'), which were selected based on their indicator values of soil moisture requirement, forage quality and tolerance towards grazing (Dierschke and Briemle 2002), as well as *Trifolium repens* L. ('Rivendel'), *Lolium perenne* L. ('Signum') and *Dactylis glomerata* L. ('Donata') as controls. The species were established in summer 2009 as pure stands in containers as a randomized block design with four replicates (64 containers of 30 L, filled with a homogeneous mixture of 20 kg air-dried sand, 5.5 kg air-dried compost soil and 0.9 kg vermiculite with a top layer of 1.5 kg compost as a seed bed). In spring 2010, *L. uliginosus* was re-sown due to total frost damage in winter.

The relation of volumetric soil water content (regular weighing) and soil water tension was determined as a soil water retention curve with a pressure plate extractor. For the control treatment, the containers were re-watered to a water content of 25 vol. % (-0.03 MPa) once it dropped below 18 vol. % (-0.3 MPa). Water limitation was imposed during three periods in spring 2010 (moderate limitation), summer 2010 and spring 2011 (both severe limitation). For the moderate water limitation, the containers were left unwatered until three days after the first sward type (*T. repens* in most cases) had reached a soil water content of 10 vol. % (-1.5 MPa), then re-watered to 25 vol. % and left to fall dry again in the same way a second time. For the severe water limitation, the containers were left unwatered until five days after the first sward type had reached a soil water content of 10 vol. %, re-watered to 25 vol. % and left to fall dry again in the same way two more times. The total water consumption of each container during each period was recorded. Between periods of water limitation, one re-growth at non-restricted water supply was allowed for. No fertilization was carried out.

Dry matter (DM) yield was assessed by clipping the aboveground biomass at 3 cm stubble height at the end of each period. Herbage was dried at 60 °C for 48 hours and weighed. The agronomic water-use efficiency (aWUE) was calculated based on dry matter yield and total water consumption for each container. The harvested biomass was analysed for stable carbon isotope composition ($^{13}C/^{12}C$) with an isotope ratio mass spectrometer to calculate intrinsic water-use efficiency (iWUE, i.e. CO_2 assimilation per stomatal conductance) according to Farquhar *et al.* (1989).

An analysis of variance was calculated to determine the effects of the factors plant species and level of water supply on dry matter yield and water consumption within a period of water limitation.

Results and discussion

Water limitation significantly ($P<0.001$) decreased DM yields, but the extent of yield reduction differed distinctly among the tested species. Under water limitation, *T. repens* produced merely about half the yield of the control, whereas *M. falcata* and *M. lupulina* on average reached ≥ 60% of the yield of the control. In the tested grass species, yields under water limitation were > 80% of those of the control (Table 1).

Agronomic WUE, on average, was higher in the *Medicago* ssp. and in *T. repens* than in the grasses. Values of this parameter decreased for most species at soil water content values < 10% (Figure 1, left). Soil water content was not as strongly correlated with aWUE as with iWUE. Intrinsic WUE consistently increased in all species with decreasing soil water content (Figure 1, right). The iWUE of *M. lupulina* and *T. repens* was comparatively large across the tested range of soil water content, whereas *M. falcata* and the grass species consistently showed comparatively small iWUE values. The differences in enrichment of ^{13}C in the harvested biomass among the tested species may indicate different strategies, e.g. concerning the extent of stomatal closure at water limitation. In legumes, N fixation may explain higher WUE values as compared to grasses. Our data hint at strong inter-specific differences in WUE for the tested legume species that warrant further consideration.

The results of this experiment have pointed out higher dry matter yield stability than in *T. repens* under conditions of severe water limitation for some legume species, and particularly for *M. lupulina*. This species also featured high values both of aWUE and iWUE. We therefore suggest further research on this species as an alternative to *T. repens* in pasture swards in response to predicted future climate change involving periods of limited precipitation in summer.

Further research should additionally examine the contribution of the potential alternatives to *T. repens* regarding dry matter yield and forage quality in grass-clover mixed stands.

Table 1. Mean dry matter (DM) yield and mean total water consumption of pure stands of six legume and two grass species under conditions of severe water limitation (4 to 10 vol. % H_2O in soil) and in the control treatment (18 to 24 vol. % H_2O in soil). Data shown are means of two periods of severe water limitation in summer 2010 and spring 2011. Within columns, values followed by the same letter do not differ significantly at the $P = 0.05$ level.

Species (abbreviation)	DM yield [g/container]		water consumption [l/container]	
	water limited	control	water limited	control
L. corniculatus (Lc)	46.0 c	83.0 b	19.8 b	28.2 bc
L. uliginosus (Lu)	43.5 bc	84.6 b	19.5 b	27.4 bc
M. falcata (Mf)	44.6 c	70.1 ab	18.8 ab	21.3 ab
M. lupulina (Ml)	51.6 c	84.3 b	18.2 ab	22.6 ab
O. viciifolia (Ov)	32.0 ab	56.9 ab	17.0 a	22.9 abc
T. repens (Tr)	48.0 bc	94.9 b	19.8 b	29.9 c
D. glomerata (Dg)	33.2 a	35.5 a	19.8 b	19.9 a
L. perenne (Lp)	31.9 a	37.2 a	18.8 ab	19.4 a
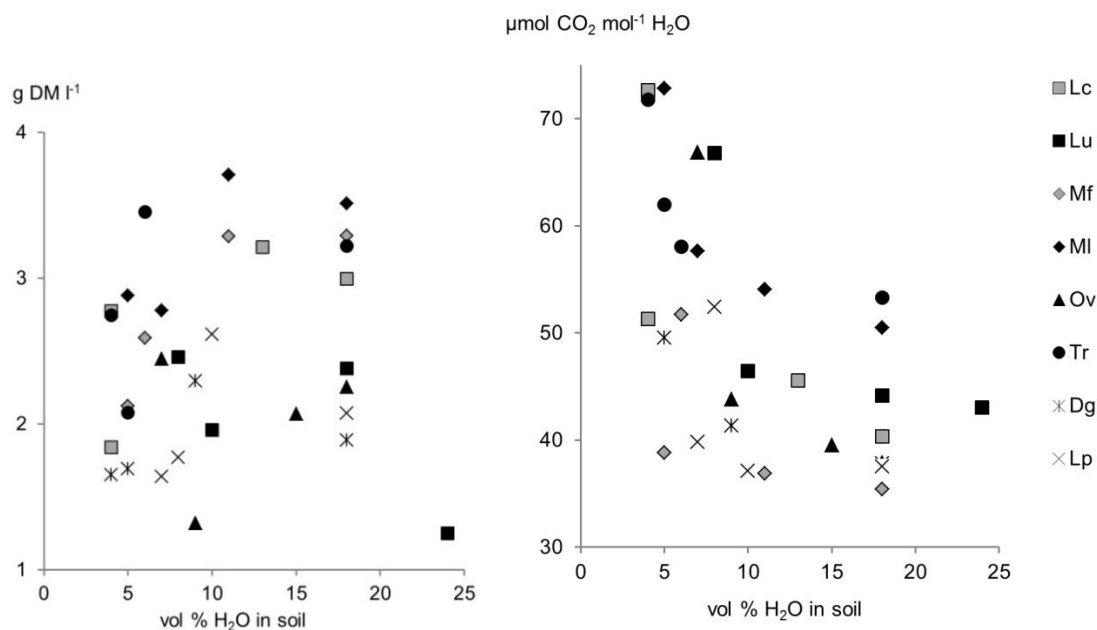
Figure 1. Agronomic (left) and intrinsic (right) water-use efficiency of six legume and two grass species in response to volumetric soil water content [%]. Shown are four values per species: three for water-limited treatments for each of the three stress periods and one for the control (averaged over three periods). Values on the x-axes are minimal values over the respective periods. For species name abbreviations see Table 1.

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Drought effects on herbage production of permanent grasslands in northern Germany

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Abstract

We investigated the influence of sward composition (dry weight by functional group) and nitrogen fertilization on the annual yields of drought-stressed meadows from three sites typical of northern Germany by generating artificial drought with rain-out shelters that held back, on average, 148 of 242 mm rainfall from the beginning of the vegetation period until the end of the last stress treatment. Yields decreased from on average 7510 to 7298 kg ha^{-1} a^{-1} when swards were exposed to drought stress, but this effect was enlarged and modified by fertilization, site and sward composition. Nitrogen-fertilization had a positive effect on productivity. The influence of sward composition was site specific and may rather have been a functional-group effect than the influence of diversity. Future productivity losses due to drought stress may be smaller than expected.

Keywords: water limitation, yield, sward composition, nitrogen

Introduction

Larger climatic variability and frequent climate extremes like drought periods will characterize northern Germany's future climate (Schär *et al.*, 2004). More frequent and severe drought events are expected to have a negative impact on herbage production and fodder quality of permanent grassland (Beierkuhnlein *et al.*, 2011). We hypothesize a negative effect of drought stress on herbage yield and mitigating influences of site, sward composition and fertilization on the size of its effect. In particular, the supposedly positive influence of diversity on grassland productivity (Tilman *et al.*, 2001) still remains unclear where agriculturally managed permanent grasslands are concerned.

Materials and methods

In a three-year experiment we investigated the effects of drought (with and without rain-out shelters), sward composition (with and without reduction of dicot-species cover) and nitrogen fertilization (with 90 kg ha^{-1} or without) in a completely randomized block design with four replicates at three locations. Spring and summer drought events (five to six weeks long) were induced by installing rain-out shelters, each of 3.24 m², on three different grassland sites typical for northern Germany (south-eastern (SE) lowland (average -160 of a total 230 mm rainfall from the beginning of the vegetation period until the end of the second stress treatment), sub-mountainous (ave. - 148 of 258 mm), and north-western (NW) lowland (ave. -136 of 237 mm)). Rainfall data are averages over 2011 and 2012. Sward composition was manipulated with herbicides to reduce dicot dry matter yield share from on average 14 to 4 g kg^{-1} to measure the influence of sward composition. Biomass samples were cut after each stress period and once again in autumn on 0.16 m² and at 7 cm stubble height. Herbage production was determined as accumulated dry weight production (drying at 60 °C for 48 h) per year (three cuts/year). Data were analysed by applying generalized mixed models that allowed for heteroscedasticity. Site, year and block were treated as random effects as they are nested and do not represent real treatments.

Results and discussion

The strongest factor effects on annual dry matter production were fertilization and site identity (Table 1). All sites showed enhanced productivity after N-fertilization. Generalizing over all sites, fertilization led to a larger biomass yield under drought-stress conditions, regardless of sward composition. Unfertilized swards had smaller biomass yields if swards were diverse. Grass-dominated, unfertilized swards did not show biomass differences between drought-stress levels. The three sites reacted differently to the treatments (Figure 1). If fertilized grass-dominated lowland swards were exposed to drought stress, they had larger biomass yields than if exposed to rainfall. The unfertilized, grass-dominated lowland swards did not show any differences in yield regarding drought exposure. As fertilization leads to higher grass-tillering rates and thus higher sward density (Simon and Lemaire, 1987) that might decrease evaporation. Plants, e.g. *Lolium perenne* (Lucero *et al.*, 2000), may also be able to increase their water-use efficiency if droughts occur.
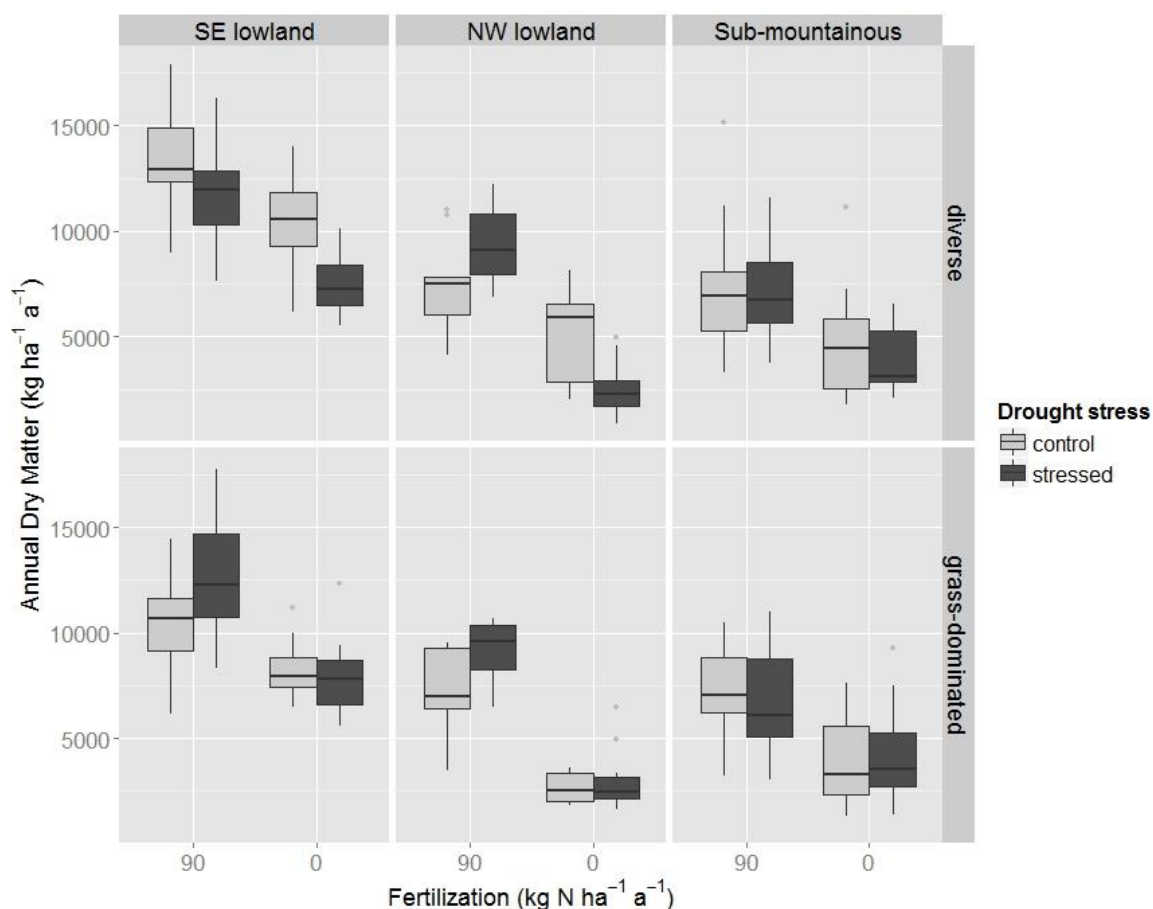
Figure 1. Productivity as annual dry matter yield (kg ha^{-1}) on three sites (upper row) under the influence of drought stress (filling), sward composition (right side) and N-fertilization (lower axis). Data are derived from three years (2011 - 2013) of observation.

The influence of diversity, however, was not found to be as important as suggested by reports in the literature (e.g. Tilman *et al.*, 2001). The two diverse lowland swards showed dissimilar responses to drought stress, with a site-specific influence of fertilization (see Figure 1). The sub-mountainous swards did not show differences in yield regarding sward composition and drought stress. Sward type might only be influential if the dicot share in the biomass exceeds a certain threshold: our diverse sub-mountainous swards did not exceed eight forb species that delivered a mean share of 42 g kg^{-1} of annual biomass (data not shown). The lowland swards had a higher dicot share (average 50 - 100 g kg^{-1} of dry matter), even if grass-

dominated. All trends seen in the annual yields (Figure 1) are already showing in the summed yields of the first two cuts (after the stress periods). That means the swards did not show compensational growth after the stress periods (data not shown) and all results of annual dry matter yield can be attributed to the first two cuts. This suggests a strong plasticity and high resilience to drought stress (as most swards did not show reduced biomasses if stressed), or soil water content was not the limiting factor, although soil water measurements (data not shown) at the densely rooted upper soil level (15 cm) indicated drought. Further works will clarify this.

Table 1. Anova type III table from Linear Mixed Model of annual dry matter yield (kg ha^{-1} a^{-1}) from three sites over 3 harvest years. The not significant (n.s.) variables were left in the model because omitting them deteriorated the model fit (unlike all other n.s. variables).

Factor		F	Degrees of freedom	P	Significance level
Sward composition	(s)	1.62193	1	0.2039	n.s.
Fertilization	(f)	35.36478	1	< 0.001	***
Drought stress	(d)	2.50795	1	0.1145	n.s
Year	(y)	6.54821	1	0.0111	*
f × site		6.40737	2	0.0019	**
y × site		28.24634	2	< 0.001	***
s × d		18.40618	1	< 0.001	***
f × d		11.72089	1	0.0007	***
y × d		2.51952	1	0.1136	n.s.
s × y		1.62961	1	0.2029	n.s.

Conclusion

Future productivity losses due to drought stress might be smaller than expected, depending on the site conditions. Fertilization seems to have a supporting effect on drought resilience of lowland swards. Dicot richness can have a stabilizing and yield improving function as mentioned in literature (Tilman *et al.*, 2001), but the dicot share of the biomass maybe needs to exceed a certain threshold to deliver benefits to productivity.

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The effect of drought on the depth of water uptake of deep- and shallow-rooting grassland species

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Abstract

Increased incidence of drought, as predicted under climate change, has the potential to disrupt grassland production, highlighting the need for the design of grassland management systems adapted to future climate-change scenarios. More-deeply rooted plants are more likely to survive extended periods of drought by accessing deeper soil layers that contain higher soil moisture levels. However, very little is known about the depth of water uptake of grassland species as affected by drought. Therefore, in this study, we used the natural abundance $\delta^{18}O$ isotope method to assess the effect of drought on the depth of water uptake of two shallow-rooting (*Lolium perenne* L. and *Trifolium repens* L.) and two deep-rooting species (*Cichorium intybus* L. and *Trifolium pratense* L.) in intensively managed grassland monocultures to test the following hypotheses: 1) drought will result in a shift of water uptake to deeper soil layers and 2) deep-rooting species take up a higher proportion of water from deeper soil layers relative to shallow-rooting species. The $\delta^{18}O$ isotope method showed a large treatment effect on the proportional contribution of the 0-10 cm soil depth interval to plant-water uptake, which ranged from 0.08 to 0.82. As hypothesized, water uptake shifted to deeper soil layers under drought conditions, with the exception of *T. pratense* in monoculture. For three of the species, the depth of water uptake corresponded to the rooting depth classification, but the deep-rooting *T. pratense* actually showed reliance on shallow soil-water uptake.

Keywords: $\delta^{18}O$ natural abundance, *Lolium perenne, Trifolium repens, Cichorium intybus, Trifolium pratense*, water uptake

Introduction

Increased incidence of weather volatility and drought, as predicted to occur under future climate change, has the potential to disrupt grassland production. This highlights the need for designing grassland management systems for forage production which are adapted to future climate change scenarios. Here we focus on the plant functional trait of rooting depth as an adaptation option to drought conditions, as plants that are more deeply rooted are more likely to survive extended periods of drought by accessing deeper soil layers that contain higher soil moisture levels (Chaves *et al.*, 2003). However, very little is known about the depth of water uptake of grassland species and how this is affected by drought, because:1) most grassland research has focussed on aboveground biomass as opposed to belowground measures, and 2) the abundance of roots is not necessarily equivalent to root activity, i.e. water uptake. Increased insight into the effect of drought on the depth of water uptake of different species may yield important information with which to predict both the effect of future climate change on grassland production, and to design agricultural systems with improved resistance and resilience to drought.

The natural abundance of $\delta^{18}O$ in soil and plant water can be used to measure the depth of water uptake of individual species (Durand *et al.*, 2007). Therefore, the objective of this study was to assess the effect of experimentally induced drought on the depth of water uptake of two shallow-rooting and two deep-rooting species in intensively managed grassland monocultures by using the natural abundance $\delta^{18}O$ isotope method. We tested the following hypotheses: 1) drought will result in a shift of water uptake to deeper soil layers, and 2) deep-rooting species

take up a higher proportion of water from deeper soil layers compared to shallow-rooting species.

Materials and methods

We selected four model species of which two were shallow-rooting (*Lolium perenne* L. and *Trifolium repens* L.) and two were deep-rooting species (*Cichorium intybus* L. and *Trifolium pratense* L.). In August 2011, monocultures of all four species were sown on 3 m × 5 m plots in Reckenholz, Switzerland. In 2012, plots were cut six times and received 200 kg N ha^{-1} yr^{-1} and enough P and K as to be non-limiting for intensively managed grassland. Using rainout shelters, half of the plots were subjected to a drought treatment of 10 weeks summer rain exclusion (spanning 2 regrowth periods).

Natural abundance of δ^{18}O in soil and plant water was used to assess the depth of water uptake of individual species. The lower evaporation rate of heavy isotopes increases the concentration of ^{18}O in water at the soil surface. This results in a vertical gradient in isotopic composition of water in the soil and therefore the composition of plant xylem water is an indicator of the mean depth of water uptake (Durand *et al*., 2007). Approximately one week before the end of the drought period, stem bases (up to 1.5 cm above soil level) were collected from five to eight tillers per plot of all four sown species. At the same time and for each plot, three soil cores (2 cm diameter) were taken to 40 cm depth and divided into five segments (0-5, 5-10, 10-20, 20-30 and 30-40 cm). Water from the soil and plant samples was extracted using cryogenic vacuum distillation. Water samples were analysed for oxygen 18 isotopes at the Boston University Stable Isotope Laboratory. We applied the IsoSource stable isotope mixing model (Phillips and Gregg, 2003) to quantitatively determine the proportional contribution of each of the sources (i.e. five soil depth intervals) to the plant stem water δ^{18}O signature. All statistical analyses were carried out using the statistical software R.

Results and discussion

During the drought period, a total of 247 mm of rain was excluded from the drought plots, which corresponded to 21% of the total annual rainfall for 2012. There was a large treatment effect on the proportional contribution of the 0-10 cm soil depth interval to plant water uptake ($PCWU_{0-10}$), which ranged from 0.08 to 0.82 across the four species (Figure 1a). This means that between 8% and 82% of the total water uptake was derived from the 0-10 cm soil depth interval, respectively, and the remainder (92% and 18% respectively) was taken from deeper soil layers (10-40 cm soil depth, Figure 1b).

In line with hypothesis 1, the $PCWU_{0-10}$ of *L. perenne*, *T. repens* and *C. intybus* was reduced by 53%, 40% and 69%, respectively, under drought compared to control conditions (Figure 1a), as a result of a proportional increase in water uptake from deeper soil layers (Figure 1b). In contrast, the $PCWU_{0-10}$ of *T. pratense* increased by 33%, resulting in a significant ($P < 0.05$) species × drought interaction. The reduction in aboveground biomass under drought conditions did not appear to be related to increased depth of water uptake. In fact, the species with the deepest water uptake (*C. intybus*) was − at this experimental site − one of the species most affected by drought (Hofer *et al*., 2014; site Reckenholz). Therefore, there are probably different mechanisms that determine the resistance to drought in this system, such as species drought tolerance and nutrient availability.

As expected, the two shallow-rooting species *L. perenne* and *T. repens* relied mainly on shallow soil water ($PCWU_{0-10} = 0.53$), whereas the deep-rooting *C. intybus* relied on water from deeper soil layers ($PCWU_{0-10} = 0.16$) (Figure 1). However, $PCWU_{0-10}$ of the second deep-rooting species, *T. pratense* was, on average, 0.68, which is even higher than for the shallow-rooting species. This highlights the fact that the presence of roots is not necessarily equivalent to root

activity, underpinning the necessity of studies examining root activity. Instead, the presence of roots determines the potential access to different soil layers.
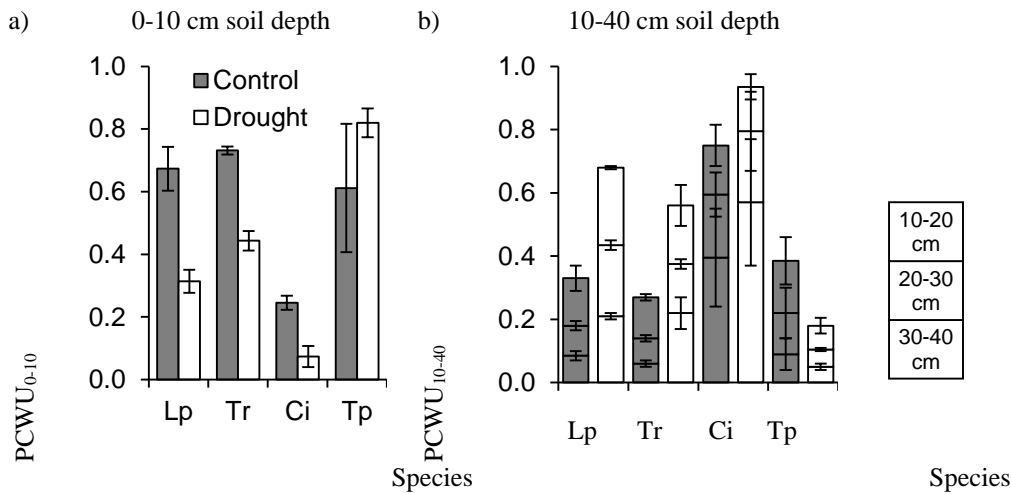
Figure 1. The proportional contribution to plant water uptake of a) the 0-10 cm soil depth interval ($PCWU_{0-10}$) and b) the 10-40 cm soil depth interval ($PCWU_{10-40}$, split up into 10-20, 20-30 and 30-40 cm soil depth intervals) for the two shallow-rooting species (Lp = *L. perenne* and Tr = *T. repens*) and deep-rooting species (Ci = *C. intybus* and Tp = *T. pratense*) under control (shaded bars) and drought (white bars) conditions. Error bars are SE, *n* = 2.

Conclusion

The natural abundance $\delta^{18}O$ technique provided novel insights into the depth of water uptake of deep- and shallow-rooting grassland species and revealed large interspecific differences and shifts in response to drought.

Acknowledgements

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CLIMAGIE: A French INRA project to adapt grasslands to climate change

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Abstract

The research teams inside the INRA project CLIMAGIE aim to improve the production of knowledge and subsequent innovations for adapting grasslands to the risks of climate change that threaten the maintenance of the ecosystem services provided by grasslands. Using plant biodiversity should contribute to improve grassland resistance and resilience under high climatic constraints and low inputs. Up to now, establishment and maintenance of pluri-specific grasslands remain poorly controlled. Collaboration between communities and functional ecologists, ecophysiologists and quantitative geneticians will provide new rules for species and cultivars ecotypes assembling. We will build up a new framework to propose a range of solutions depending on pedoclimatic conditions and grassland functions, enabling farmers and breeders to cope with uncertainties attached to future climate scenarios. That framework will be tested experimentally and *in silico* with the models under construction in our teams. It will contribute to the definition of new ideotypes and breeding schemes of major species for plant breeding, in close collaboration with seed companies and end users through participatory selection programmes.

Keywords: climate change, water status, temperature, grasses, legumes, grasslands, plant breeding, modelling

Introduction

The impacts of temperature, water deficits and CO_2 on sown monospecific grasslands have been assessed using crop models (Durand *et al.*, 2010). In most locations studied so far, grasslands might show resilience or resistance, or even produce more (though with greater irregularity) under various scenarios of climatic conditions including increase of CO_2. However, the responses of plants to extreme droughts and heat waves are not well described in the current models. The future management of grasslands will be based on lower inputs (fertilization, water) requiring the use of more genetically diverse grasslands that can use resources more efficiently (Darwin, 1859; Huyghe and Litrico, 2008). There has been less investigation so far of intra-specific genetic variability, despite clear evidence for of importance (Sampoux *et al.*, 2010; Poirier *et al.*, 2012). Hence, both the ranges of climate conditions and genetic variability must be explored more deeply. Phenology and plant productivity responses to water, temperature and nitrogen in particular need to be re-assessed over the full ranges projected in the future.

The project

The multidisciplinary INRA research programme CLIMAGIE organizes the collaboration between ecologists and quantitative geneticians to provide new rules for assembling species and cultivar ecotypes. CLIMAGIE builds up a framework to propose a range of solutions depending on pedoclimatic conditions and grassland functions, enabling farmers and breeders to cope with uncertainties attached to future climate scenarios. Mediterranean conditions with harsh water deficits and frequent heat waves bring about a complete cessation of plant growth during summer. That situation contrasts with temperate conditions, where summer conditions allow for a minimum grassland production. A recent study suggested that the summer water balance (P-ET°) would be a relevant indicator to ascribe a particular region to one of these two domains (Poirier *et al.*, 2012). Each type illustrates tradeoffs between ecophysiological structures and functions (Volaire *et al.*, 2013). The intraspecific genetic variability of most important traits associated to each type are investigated in CLIMAGIE, especially phenology, vegetative shoot and root growth, spatial and temporal patterns of water extraction, water use efficiency (δ^{13}C in foliage biomass), nitrogen fixation and absorption, fructan concentrations and their responses to high temperatures and water deficits. These responses are integrated under the conditions of dense swards, with intense competition for light, both *in vivo* using controlled experiments and *in silico* using models under construction in our teams. This contributes to the definition of new ideotypes and breeding schemes for major species (*Lolium perenne, Festuca arundinacea, Dactylis glomerata, Trifolium pratense* and *Medicago sativa*), in close collaboration with seed companies on the one hand, and directly with end users through participatory selection programmes on the other hand. Three integrated groups of tasks are defined, at the relevant levels of complexity, both in terms of objects and methods (Figure 1.) The tasks of the project are organized within three work packages:

1. *Analysis of the genetic intra- and inter-specific variability of the physiological responses to temperatures and droughts in grassland species (legumes and grasses).* In particular, the morphogenetic response of various populations in important grassland species to the full range of temperature (5-40 °C) is studied. The capture of water is investigated using (i) the relationships between root-system architecture and the drought resistance of populations, (ii) a new interpretative model of δ^{13}C variations in the foliage of diverse cultivars growing in the same conditions, and (iii) water control and measurement facilities to relate whole plant surface temperatures to soil-plant water relations. The evolvability of grass populations under severe drought conditions was studied in *Festuca arundinacea* and *Dactylis glomerata* and the potential of such conditions to select elite populations is investigated. Integrated methodologies enable the genetic variability of water use, water use efficiency and summer dormancy to be tested. All these studies involve multidisciplinary research groups.

2. *Modelling of the dynamics of the long-term production of sown grasslands.* Three models will be tested for: (i) spatially explicit tillering of multispecies grass swards based on the SisFRT model (Lafarge and Durand, 2010), (ii) individual based competition including legumes and grasses, and (iii) simulation of sexual reproduction and transmission of traits to the next generation within swards.

3. *Operational selection schemes, ideotypes.* This includes (i) novel methodologies to assess and manage both *ex situ* and *in situ* genetic resources including biogeographical approaches to assess the presence of ecotypes in relation to local pedo-climatic conditions, and (ii) designing selection procedures for mixed-species sown grasslands.

The project's results will be presented at a meeting in autumn 2015 in Lusignan, France.
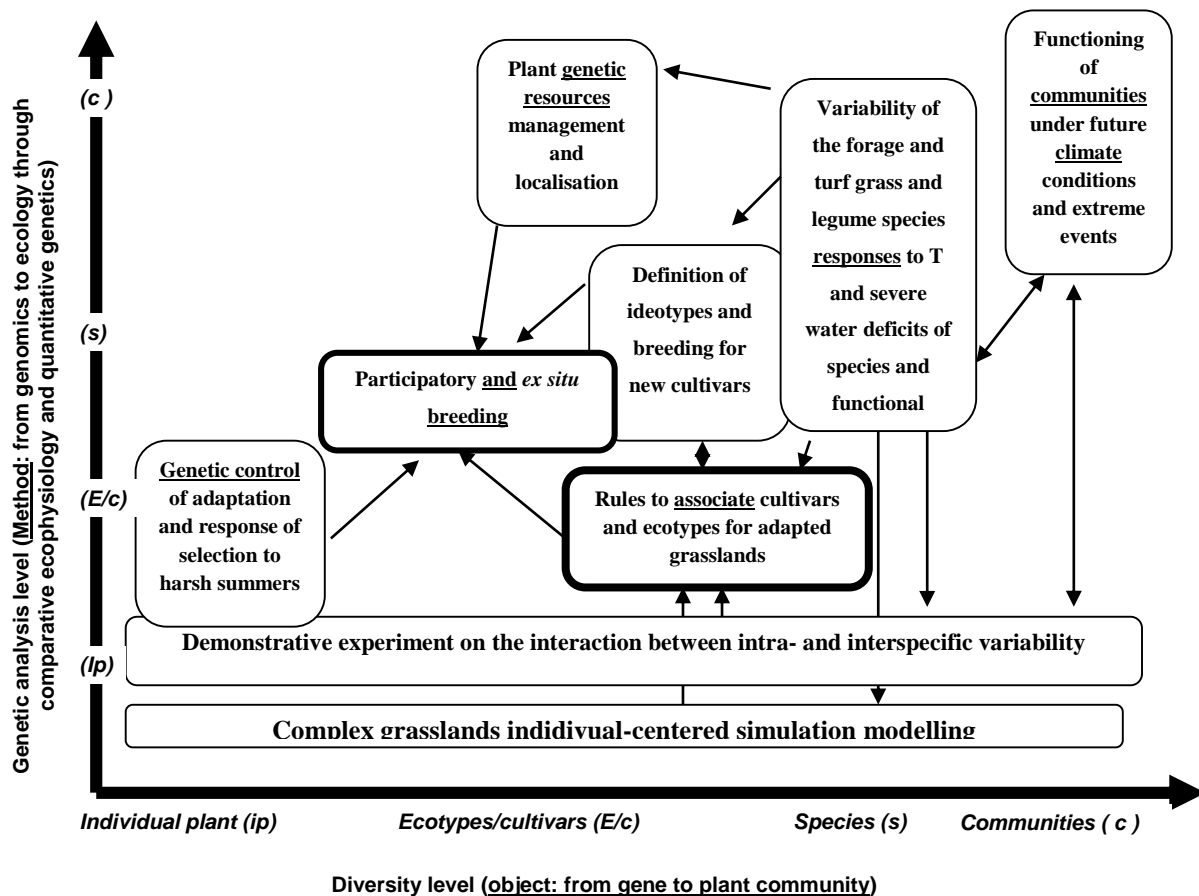
Figure 1. Description of the different groups of tasks in CLIMAGIE. (Arrows indicate the exchange of information between the tasks.)

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Comparison of temperature responses of different developmental processes in *Medicago sativa* L. and *Festuca arundinacea* Schreb.

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Abstract

Temperature is one of the most important factors affected by climate change and is also one of the most important variables involved in the control of plant developmental processes. We measured germination, leaf appearance, coleoptile expansion, radicle expansion and leaf expansion rates in lucerne (*Medicago sativa* L.) and tall fescue (*Festuca arundinacea* Schreb.). All processes studied related to plant development, and expansive growth followed common Arrhenius-type responses curves on the wide range of temperature (5–40 °C) in each studied species. This result has important consequences for modelling of temperature effects associated with global changes.

Keywords: temperature, development, growth, lucerne, tall fescue

Introduction

Temperature is one of the most important factors affected by climate change. The Intergovernmental Panel on Climate Change anticipates an increase of the global average temperature from +1.8 °C (1.1–2.9 °C) to +4.0 °C (2.4–6.4 °C) by 2100 (IPCC, 2007). Moreover, temperature is one of the most important variables involved in the control of plant developmental processes (i.e. plant phenology, organogenesis and expansive growth). Hence, the productivity and quality of grassland are expected to change in the near future in response to changes in climate (Brisson and Levrault, 2010; CLIMFOUREL, 2011; Ruget *et al.*, 2013). In a context in which climate changes could hasten growth in spring and increase the risk of summer yield losses, it is essential to study more thoroughly the effects of temperature on developmental processes of pasture species.

For three crop species, Parent and Tardieu (2010) showed in a meta-analysis of 12 literature references, that the germination rate, cell division rate, leaf initiation and appearance rate, leaf expansion rate, seedling expansion rate and the reciprocal of the duration of phonological phases followed a common Arrhenius-type response curve to temperature after normalization within each species. In the case of perennial pasture species, which have not been subjected to a long history of varietal selection, such a result has to be confirmed. The present study addresses this question on a temperate grass (*Festuca arundinacea* Schreb.*,* tall fescue) and a legume (lucerne; *Medicago sativa* L.) species of broad geographic dispersion. Our objective is to analyse the short-term response curves of developmental processes (Table 1) for *M. sativa* (cv. Barmed and cv. Harpe) and *F. arundinacea* (cv. Soni and cv. Centurion) in the wide range of temperature 5–40 °C.

Materials and methods

Germination: After wet-stratification for *F. arundinacea* or scarification treatments for *M. sativa*, seeds were placed at constant temperature (from 5 to 40 °C) and darkness in germination chambers in 90 mm diameter Petri-dishes, on two sheets of Whatman (#3645 WM-France) paper imbibed with 5mL of deionized water. Seeds were monitored regularly.

Coleoptile and radicle expansion rate: After wet-stratification or scarification treatments, seeds were placed in germination chambers at 25 °C and darkness. When the radicle or coleoptile length was >1mm, seeds were placed in growth chambers over sheets of blue blotter-paper at constant temperature (from 5 to 40 °C), high humidity, darkness and regularly irrigated

by deionized water. Using a digital camera, photos of the seeds were taken routinely. Coleoptile and radicle expansion rates were determined by image analysis. Three replicates of 30 seeds were used for each population at each temperature.

Leaf appearance, stem expansion and leaf expansion rate: Experiments were conducted in growth chambers in fertirrigated pots. At first, an excessive number of plants are grown at 25 °C for 3 weeks. Then, they go through a selection process to keep a homogenous set of plants (i.e. 4.5–5.5 leaves for *M. sativa* and 2.5–3.5 leaves for *F. arundinacea*). The homogenous selection is then transferred to the studied temperatures (5, 10, 15, 20, 25, 30, 35 or 40 °C) with a moderate light intensity (400 to 500 µmol.m^{-2}.s^{-1}), a constant photoperiod (16h) and a low water vapour saturation pressure (<1.5 kPa). Regular measurements (Table 1) were performed on plants with a ruler. Measurements stopped at 10 leaves for *M. sativa* and at 6 leaves for *F. arundinacea*. Results are presented for temperature treatments at 10, 15, 20, 25, 30 and 35 °C.

Table 1. Developmental processes presented in this study

Process	Measurements
Plant development	Germination rate
	Leaf appearance rate
Expansive growth	Coleoptile expansion rate
	Radicle expansion rate
	Stem expansion rate
	Leaf expansion rate

Results and discussion

All processes studied related to plant development (germination rate, leaf appearance rate) and expansive growth (coleoptile and radicle expansion rate, stem expansion rate, leaf expansion rate) followed common Arrhenius-type responses curves on the wide range of temperature (5–40 °C) in each studied species (Figure 1A, 1B and 1D). In order to allow comparison between processes, data were normalized by the mean rate at the optimum. However, different responses to temperature can be observed between species. Plants of *F. arundinacea* have an optimum of development around 25 °C whereas for *M. sativa* this is round 30 °C (Figure 1C). Because we worked with populations, standard deviations were important especially at high temperatures.

Conclusion

In line with the results of Parent and Tardieu (2010), the results presented here suggest that a coordination might exist between developmental processes via a common response curve to temperature in each of the species studies. This result has important consequences for the breeding and modelling of temperature effects associated with global changes. Further processes are currently being analysed, including cell division, photosynthesis and nitrogen fixation.
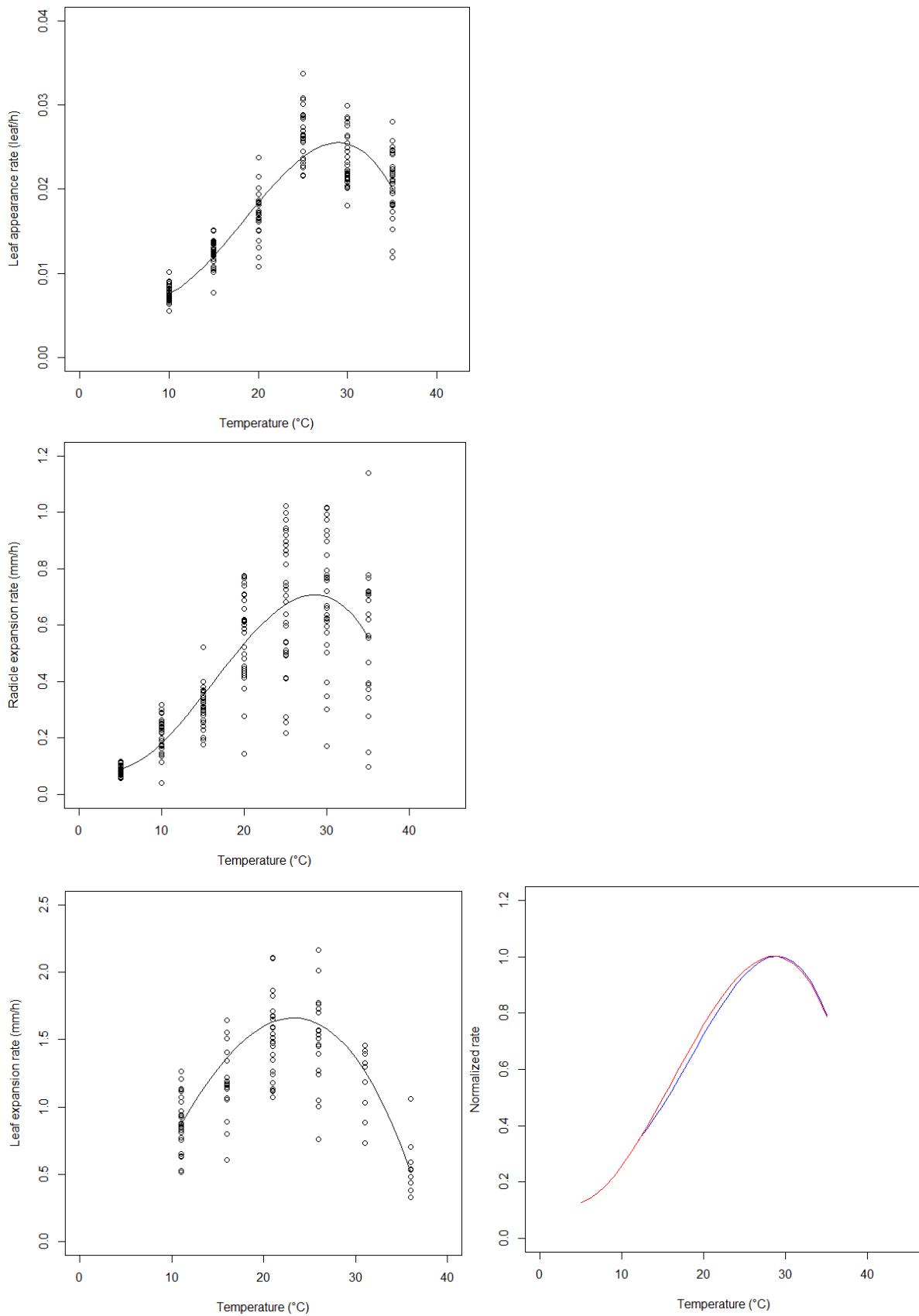
Figure 1. Figure 1A, Reponses of leaf appearance rate to temperature (cv. Harpe). Figure 1B, Reponses of radicle expansion rate to temperature (cv. Harpe). Figure 1C, Reponses of the leaf-4 expansion rate to temperature (cv. Centurion). Figure 1D, Normalized rates of responses to temperature of leaf appearance (10–35 °C) and radicle expansion rate (5–35 °C). Just normalized responses to temperature are presented here.

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Qualitative overview of mitigation and adaptation options in livestock systems

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Abstract

During the last decades the effects of climate change have received a lot of attention. Many mitigation options have been tested and more recently these are studied in an integrated manner with adaptation options in the FP7-funded project AnimalChange. This paper provides a qualitative overview of mitigation and adaption options in livestock systems at the level of manure/fertilizer, soil, feed/crop and animal, and of their synergies and trade-offs between individual greenhouse gases (GHG). Many of these options are linked to grasslands.

Keywords: adaptation, mitigation, synergies, trade-offs

Introduction

During the last decades the effects of climate change have received a lot of attention. The sources and sinks of GHG emissions have been identified and the variation in their size has been evaluated. Many mitigation options have been tested experimentally and the results have been documented in several reviews (e.g. Vergé *et al.*, 2007). Models are developed to predict GHG emissions and to evaluate mitigation strategies. In a similar manner, adaptation options have been studied (e.g. Olesen *et al.*, 2011) which is particularly important for areas that are most vulnerable to climate change. Research results show numerous interactions between mitigation and adaptation in the context of different environmental and socio-economic conditions. Generally, limited information is available on the quantification and comparison of synergies and trade-offs however, and few papers report on this (e.g. Smith and Olesen, 2010). The project AnimalChange aims to provide scientific guidance on the integration of adaptation and mitigation objectives and to design sustainable development pathways for livestock production in Europe, in northern and Sub-Saharan Africa and Latin America. The aim of the present study was to provide a qualitative overview of mitigation and adaptation options in livestock systems at the level of manure/fertilizer, soil, crop/feed and animal, and of their synergies and the trade-offs between individual GHG.

Materials and methods

The overview of mitigation and adaptation options and their interaction is presented as a matrix. It is based on a review of available literature, expert judgement and additional information provided by the project partners of AnimalChange (e.g. recent research which has not yet been published, information from other climate change related European projects). AnimalChange partners represent various countries in Europe, Africa and Latin America. The overview focuses on livestock production systems.

Results and discussion

Table 1 provides a qualitative overview of the most relevant mitigation and adaption options and of their synergies and trade-offs between GHG. The options are strongly linked to changes in the N and C cycles of the farming system. Four categories of options are distinguished in this paper: at the level of manure/ fertilizer, soil, crop/ feed and animal. Many options are linked to grasslands. Furthermore, there are many synergies and trade-offs between adaptation options and mitigation options. The effects of climate change may cause a reduced efficacy or applicability of mitigation strategies.

Table 1. Qualitative overview of mitigation and adaption options in livestock systems at the level of manure/fertilizer, soil, feed/crop and animal, and their synergies and trade-offs.

Option	Effects on mitigation (+)	Effects on adaptation (+)	Mit.pot. CH4 (-/+/++)	Mit.pot. N2O (-/+/++)	Mit.pot. CO2 (-/+/++)	Mitigation variability (variable/robust)	Importance in mitigation (low/high)	Adaptation variability (variable/robust)	Importance in adaptation (low/high)	Productivity impacts (-/+)	Costs (high/low/?/benefit)	Future measure / ready to use	Applicability by farmers (easy/difficult)	Acceptability for farmers (poor/good)
MANURE / FERTILISER														
Fertilisation rate	+	+	-	++		rob	high	rob	low	+	ben	ready	dif	good
Fertiliser type	+			+		var	high			+	ben	ready	dif	good
Fertiliser application	+			+		rob	high			+	ben	ready	dif	good
Cover slurry stores/manure heaps	+		++	-	+	rob	low				low	ready	easy	good
Manure cooling	+		+			rob	low				high	future	dif	poor
Manure treatment	+		++	-	+	rob	high				?	ready	dif	good
Filtering CH4 from barns	+		+	+		var	low				high	future	dif	poor
SOIL														
Reduced/zero-tillage	+	+		-	++	rob	high	rob	high		low	ready	dif	poor
Prevent soil compaction	+			+	+	var	low			+	low	ready	dif	good
Water management	+	+		+	+	var	low	var	low	+	low	ready	dif	good
Irrigation		+		+		var	low	var	low	+	low	ready	dif	good
Restoring degraded lands	+	+		+	++	rob	high	rob	high	+	high	ready	dif	poor
Pasture reclaiming/recovery	+				++	rob	high				low	ready	easy	good
Incorporation crop residues		+		-	+	rob	low	rob	low	+	?	ready	easy	poor
CROP / FEED														
Crop rotation	+	+	+	+	++	rob	low	var	low		low	ready	easy	good
Perennial crops	+			+	+	rob	low				low	ready	easy	good
Legumes and mixtures	+		-	++	+	rob	low			+	ben	ready	easy	good
New pasture species	+	+				var	low			+	low	future	dif	good
Improved crop varieties	+		+	+		var	low			+	low	future	dif	good
Novel crops	+	+	+			var	low	var	low		low	future	dif	poor
Cover crops	+	+		+	++	rob	low	rob	low	+	low	ready	easy	good
Conversion to grass	+			++		rob	low				low	ready	easy	good
Reforestation	+			+		var	low			-	high	ready	dif	poor
Optimal forage management	+		+		+	var	low			+	ben	ready	easy	good
Biodiversity		+						var	high	+	low	ready	easy	poor
Plant breeding		+						rob	high	+	high	future	easy	good
Use climate forecasting		+						var	high	+	ben	ready	easy	good
Different planting dates		+						var	low	+	low	ready	easy	good
Conservation as a buffer		+						rob	low		low	ready	easy	good
Mixed versus single species grass		+						var	low	+	low	ready	easy	good
Agroforestry		+			++	rob	high	rob	low		high	ready	dif	poor
Optimal grazing	+	+			+	var	low	var	high	+	ben	ready	dif	good
Increased feed digestibility	+		++			rob	high			+	high	ready	dif	good
Feed analysis	+		+	+		rob	low				high	ready	easy	good
Improving roughage quality	+		+			rob	high			+	low	ready	dif	good
More concentrates	+		+	+	-	rob	high			+	high	ready	easy	good
Improving grass quality	+		+			rob	high			+	low	ready	dif	good
Use of silage maize	+		++		-	rob	low				low	ready	easy	good
Additives in general	+					var	low				high	future	easy	poor
Additive nitrate	+		++			rob	low				high	ready	easy	poor
Matching supply and demand	+		+	+		rob	high			+	ben	ready	dif	good
Supplemental feeding		+	+			var	low	rob	high	+	high	ready	easy	good
ANIMAL														
Rumen control via breeding	+		+			var	low				high	future	dif	poor
Immunological control	+		+			var	low				high	future	dif	poor
Less consumption animal products	+		++	+		rob	low					ready	easy	poor
Increased production in general	+		++	+		rob	high			+	ben	future	dif	good
Incr prod extensive systems	+		++	-		rob	high			+	ben	ready	easy	good
Incr prod intensive systems	+		+			rob	high			+	ben	future	dif	good
Animal breeding	+	+	+	+		var	low	var	high		high	ready	dif	good
Animal management	+		+			var	low				ben	ready	dif	good
Animal manipulation	+		+	+		var	low				high	ready	easy	poor
Replacement rate cattle	+		++	+		rob	high			+	ben	future	dif	good
Cooling of animals		+						rob	high		high	ready	easy	good
Livestock mobility		+						var	high		low	ready	dif	good
Animal health		+						var	low	+	low	future	dif	good

It may lead to lower yields due to elevated temperatures and fluctuations in water availability. The interactions between food production, adaptation options and mitigation options are complex and often dependent on the local situation and detailed aspects of the farming system. This limits the applicability of generic results to analyse specific farming systems for example. There are also many constraints for implementation of options in agriculture (e.g. Smith *et al.*, 2007; Smith and Olesen, 2010). Mitigation and adaptation options need, therefore, to be tailored to specific regional contexts. Furthermore, in many countries, especially in Africa, the impact of agriculture on climate is an issue that is of far less importance, because of socio-economic reasons such as, for example, addressing famine (Vergé *et al.*, 2007).

A clear understanding of the consequences of options at field and animal scale is important because farmers have to make their day-to-day decisions at those scales. Simultaneously, it is important to have predictions of the synergies and trade-offs between GHG that are sufficiently accurate. Furthermore, it is important to realize that regional and global effects and decisions at the scale of field and animal affect each other. For example, the impact of rising food demands means, other things remaining equal, that a reduction in food production in a certain region would result in increased food production elsewhere.

Within the project AnimalChange, the effects of mitigation and adaptation options will be studied to quantify the consequences of adaptation and mitigation options for a range of farm systems and regions. This will lead to a consolidated overview of tested mitigation and adaptation options, including the investigation of breakthrough options and their applicability range in terms of farming systems and agro-ecological zones and their net effect on productivity and GHG emissions.

Conclusion

Many adaptation and mitigation options are linked to grasslands. Since synergies and trade-offs between GHG exist for adaptation and mitigation options, accurate predictions of the effects of these options are needed to tailor them in the context of specific farming conditions.

Acknowledgements

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Genetic diversity of *Lolium perenne* L. in the response to temperature during germination

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Abstract

Perennial ryegrass (*Lolium perenne* L.) is the major grass forage species grown in temperate regions worldwide. Temperature is one of the major factors controlling seed germination and, in the context of global climate change, breeding *L. perenne* varieties adapted to new ranges of temperature could be necessary. The objective of the work presented here was to analyse the genetic variability of perennial ryegrass in response to temperature during germination. It was observed that the responses of the ryegrass populations showed statistically significant differences ($P<0.05$). At least three groups of populations can be distinguished. The findings of this study suggest that high genetic variability exists within *L. perenne* for response to temperature during germination. This variability could be exploited to breed new varieties adapted to the new environmental conditions induced by global climate change.

Keywords: breeding, grasslands, ryegrass

Introduction

Perennial ryegrass (*Lolium perenne* L.) is the major grass forage species grown in temperate regions worldwide. In Europe, grasslands cover at least 30% of the 160 Mha Agricultural Surface Area (ASA). In France, since 2006, grasslands cover 20-25% of its continental land area, which represents around 40% of its ASA. The agricultural use-value of grasslands depends both on the structure of their canopy and on their botanical composition. The annual time course of temperature is one of the most important factors affected by climate change. Further, using a number of scenarios, the Intergovernmental Panel on Climate Change anticipates an increase in global average temperature, in the range 1.8 °C to 4.0 °C, by 2100 (IPCC, 2007). Temperature is one of the major factors controlling plant developmental rates (i.e. plant phenology, organogenesis and expansive growth). Temperature is also important in controlling seed germination. It is well documented that maximum cumulative germination is highly temperature dependent (Bewley and Black, 1994). However, the responses of ryegrass to germination temperatures have only been described for a few populations and in a narrow range of temperatures. In the context of global change, breeding ryegrass that is adapted to new ranges of temperature could be necessary. Knowing the variability of responses to temperature by different accessions of *L. perenne* germplasm is an unavoidable first step towards such breeding. Thus, the objective of the work presented here was to analyse the genetic variability of ryegrass in response to temperature during germination.

Materials and methods

Eight populations of ryegrass were evaluated. Six were wild populations collected in different places in France (Table 1). The other two were varieties obtained by divergent selection at INRA-UR4 P3F, Lusignan, France. Seeds were obtained from the Centre de Ressources Génétiques des Espèces Fourragères (INRA-UR4 P3F, France) where they were conserved at 5 °C and 30% Relative Humidity (RH). The seed dry weight was recorded from four replicates of 100 kernels before a cold stratification treatment for seven days, intended to release dormancy. After stratification, four plastic Petri-dishes (90 mm diameter) with 100 seed each, were placed in darkness in growth chambers at the following constant temperatures: 5, 10, 15, 20, 25, 30, 35 or 40 °C. Germination counting was carried out at variable time intervals and

duration that depended on the temperature treatments. A seed was considered as germinated when its radicle or coleoptile was at least 2 mm out of the seed. Here, we report data on maximum germination percentage. For each population, a third degree polynomial was adjusted by the least squares method. Sequential ANOVA pairwise comparisons were performed between the best fit of a given population and the rough data of a second one. The probability of a calculated F value greater that a tabular F (Pr>F) was calculated and a comparison matrix was constructed. The optimal temperature for germination was estimated.

Table 1. List of six wild populations of *Lolium perenne* L. collected in different sites in France and two varieties (P19 and H1) obtained by divergent selection at INRA-UR4 P3F, Lusignan, France. Information of the collection sites is included

Populations	Collection site	Altitude	Latitude and Longitude	Mean temperature of warmest quarter (°C)	Mean temperature of coldest quarter (°C)	Precipitation of warmest quarter (mm)	Precipitation of coldest quarter (mm)
ACVF10214	Nailloux	200	43° 21' 20" N 1° 37' 27" E	20.0	5.2	170	185
ACVF10491	Bésignan	650	44° 19' 13.6" N 5° 19' 31" E	18.3	2.6	171	202
ACVF20010	Bosdarros	230	43° 12' 35" N 0° 21' 41" W	18.4	5.1	188	241
ACVF50013	Saulieu	320	47° 16' 46" N 4° 13' 44" E	16.7	1.4	226	229
ACVF50039	Lure	400	47° 41' 0" N 6° 30' 0" E	17.5	1.5	259	250
ACVF60016	Reims	140	49° 15' 0.2" N 4° 2' 0" E	17.6	2.7	175	149
P19	Le Chêne	144	46° 24' 10" N 0° 4' 48" E	18.3	4.4	162	226
H1	*idem*	*idem*	*idem*	*idem*	*idem*	*idem*	*idem*

Results and discussion

The novelty of this work comes from the wide range of temperatures evaluated (5 to 40 °C). Striking results show that no germination at all was observed at 40 °C for any of the eight populations under study. Thus, values of zero recorded at this temperature were excluded from the curve fitting. It was observed that the responses of the ryegrass populations showed statistically significant differences (*P*<0.05). Indeed, the shape of the best fits were different (Figure 1). At least three groups of populations can be distinguished. The first group is formed by two wild populations and the two varieties (ACVF10214, ACVF10491, P19 and H1). For this group, there was little effect of the extreme temperatures (5 and 35 °C) on the maximum germination percentage. The third degree polynomial fitting the data could be replaced by a parabola. The second group includes three populations (ACVF20010, ACVF50013 and ACVF50039). The shape of the curve is rather asymmetric showing little effect of low temperature (5 °C) and optimum temperatures for maximum germination below 12 °C. The response of population ACVF60016 is very different from the other two groups. The asymmetry of the curve is the opposite of that the second group. Germination was poor at low temperatures (5 and 10 °C), it peaked at 25 °C, and then declined.

Overall, these results demonstrate that genetic variability exists within the *L. perenne* species, which could be exploited to breed new varieties adapted to new environmental conditions that may be induced by global climate change.
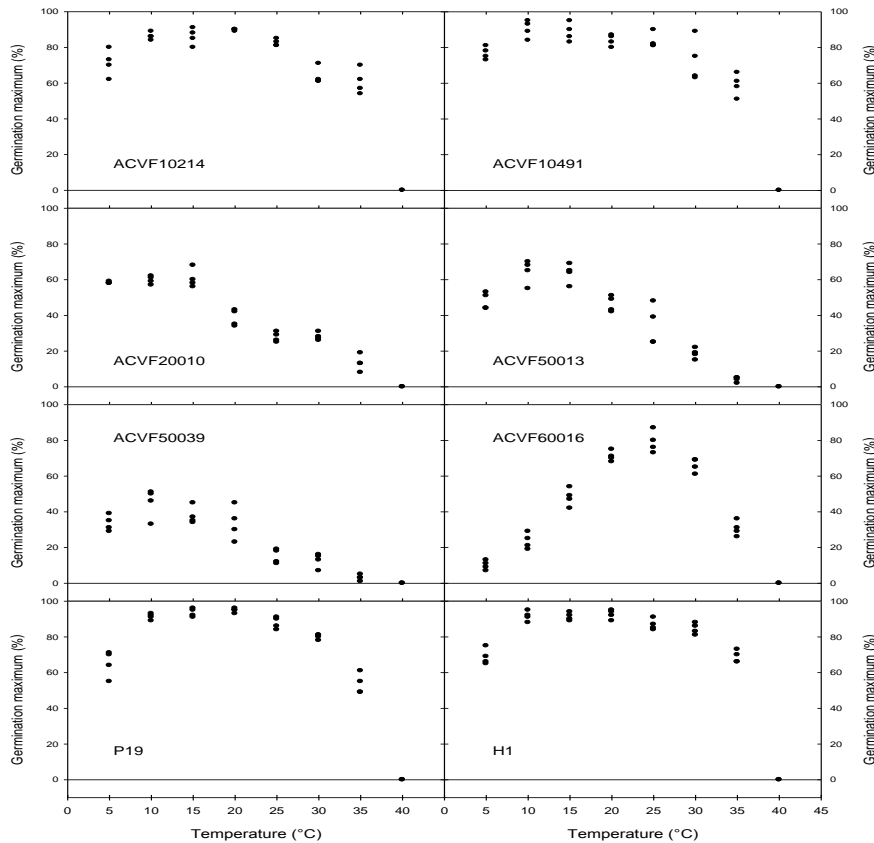
Figure 1. Maximum germination (%) of eight populations of *Lolium perenne* L. in response to constant temperature during germination.

Conclusion

The findings of this study suggest that high variability exists within the species *L. perenne* for response to temperature during germination. This should prompt physiologists to extend the analyses of response to temperature to other processes (Zaka *et al.*, 2014, this congress) and plant breeders to collect and analyse populations of ryegrass from sites with extreme environmental conditions. We suggest that seed germination of populations from northerly and cold sites is improved by high temperatures and limited by colder temperatures, and vice versa for warm-adapted populations from the South. The variability discovered in this study should serve breeders in developing ryegrass varieties for the future.

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Time of ploughing affects nitrous oxide emissions following renovation and conversion of permanent grassland

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Abstract

Grassland ploughing carries the risk of increased nitrogen mineralization and hence of increased nitrous oxide (N_2O) emissions. So far, only a few studies are available estimating N_2O-emissions after grassland ploughing at different times of the year. In the presented study we determined N_2O fluxes from autumn-ploughed and spring-ploughed grassland during two experimental years. As an alternative to grass reseeding, maize was cultivated after spring-ploughing. All treatments comprised the factor nitrogen fertilization in the form of cattle slurry with 0 and 240 kg N $ha^{-1}year^{-1}$. Results showed increased N_2O fluxes compared to the undisturbed control in all ploughed treatments. Grassland renovation in autumn resulted in highest N_2O fluxes of 1641 µg m^{-2} h^{-1} and highest cumulative emissions of 21.3 kg N_2O-N ha^{-1} $year^{-1}$. Freeze and freeze-thaw-cycles were identified as a major driver of increased N_2O fluxes during winter when ploughing of grassland had been carried out in autumn. Hence, grassland ploughing in spring induced lower cumulative N_2O-N losses reaching a maximum of 6.32 kg N_2O-N ha^{-1} $year^{-1}$ when grass or maize was sown afterwards. Cultivating maize after grassland resulted in slightly higher N_2O-N losses compared to grass resowing in spring. Fertilizer effect on N_2O-emissions was only significant after spring-time renovation. It is concluded that grassland ploughing, if necessary, should occur in spring in order to avoid additional N_2O losses due to freeze and freeze-thaw-cycle related emissions in regions where those cycles are expected. Additional N_2O losses due to cultivating maize instead of grass resowing seem to be acceptable, under favourable soil conditions, in the year of ploughing.

Keywords: nitrous oxide, grassland renovation, land use change

Introduction

Continuous intensification in dairy farming and the increased demand for renewable energy sources, such as anaerobic digestion in biogas plants, has raised the need for biomass in many parts in North-west Europe. As a consequence, permanent grassland has been converted to arable land and the management intensity of remaining grassland has been considerably increased (Taube *et al.*, 2014). In this case, grassland renovation is reported to be carried out every 5 to 10 years depending on soil type and environmental conditions (Velthof *et al.*, 2010). However, grassland renovation and conversion is usually associated with soil management practices which will ultimately lead to a loss of soil carbon, of between 10 and 32 t C ha^{-1} two years after grassland ploughing, even though grass is sown afterwards (Linsler *et al.*, 2013; Necpálová *et al.*, 2013). Regarding greenhouse gas emissions, in addition to large losses of CO_2, grassland ploughing can induce emissions of the potent GHG nitrous oxide (N_2O) (Velthof *et al.*, 2010). N_2O is a soil-born greenhouse gas which is mainly produced as a by-product and intermediate of nitrification and denitrification, respectively. Its production in soils is highly dependent on soil water content and temperature. Therefore, time of ploughing could play a key role for ploughing-related N_2O emissions due to different environmental conditions and different growth characteristics of newly established plants competing for nitrogen with soil nitrifiers and denitrifiers. In this context, grass re-seeding could be advantageously compared to other inserted forage crops because of a more rapid establishment of grass.

However, in ley farming systems, grass is usually replaced by other forage crops such as maize for a minimum of one year before grass is reseeded again.

The aim of this study was to investigate if time of grassland ploughing could affect N_2O emissions in the year following renovation, and if an inserted forage crop such as maize would increase total N_2O losses compared to prompt resowing in the year of ploughing.

Materials and methods

The field experiment was established in a long-term field trial at the experimental farm 'Lindhof' (54° 27' N 9° 57' E) of Kiel University in Northern Germany. The long-term mean annual temperature is 8.9 °C and the long term average annual rainfall is 768 mm. The soil type is classified as sandy loam (pH 5.7) with 11% clay, 29% silt, 60% sand and 1.7% C_{org} in the topsoil (0-30 cm).

Permanent grassland plots, sown in 1994, were mulched, rotovated and ploughed to a soil depth of 25 cm in September 2010, 2011 and May 2011, 2012. Ploughed plots were harrowed and re-seeded with a 30 kg ha^{-1} standard grass mixture. In addition to grassland renovation in spring, ploughed plots were sown with maize in a row width of 0.75 m with a plant density of 10 plants per m². The undisturbed sward served as control treatments. All treatments comprised the factor N-fertilizer (0 and 240 kg N ha^{-1} year^{-1} as cattle slurry). Slurry application was carried out using trailing hoses to each of the four silage cuts in the N-fertilized treatment (80, 60, 60, 40 kg N ha^{-1}). A non-N-fertilized-treatment served as control. Maize plots received the same total amount of nitrogen fertilizer shared out in three equal dressings.

Nitrous oxide emissions were measured once a week for a period of twelve months, starting shortly before grassland ploughing occurred, using the static chamber method (Hutchinson and Mosier, 1981). For N_2O measurement, pre-installed soil collars were closed with a gas-tight chamber (h=35 cm, V= 113 l) for 40 minutes and three gas samples were taken at 20-minute intervals. Samples were analysed in the laboratory for N_2O concentrations using a gas chromatograph (model 7890a, Agilent technology Inc., Santa Clara, CA, USA). N_2O fluxes were calculated for each treatment and replicate by linear regression between measured N_2O concentrations and time. The cumulative N_2O-emissions were calculated by linear interpolation of measured daily fluxes.

For statistical analysis the software R (2012) was used. The statistical model included the factors treatment, fertilizer level and year as well as all their interaction terms as fixed factors. For N_2O fluxes, soil temperature (at 5 cm depth), soil freeze-thaw-cycles, water-filled pore space and soil nitrate concentration were modelled as covariables. The plot was regarded as a random factor. Based on this model, an analysis of variances (ANOVA/ANCOVA) was used. Multiple contrast tests were conducted to compare the various levels of the influence factors.

Results and discussion

Grassland ploughing significantly increased N_2O emissions, independently of time and slurry application, compared to the undisturbed control. In contrast to authors who observed higher N_2O emissions after spring-ploughing (Velthof *et al.*, 2010), we found that soil freezing and freeze-thaw-cycles were the main driving factors for high N_2O fluxes during winter following autumn-ploughing. Hence, we observed maximum fluxes after grassland renovation in autumn in both experimental years. Significant differences in accumulated N_2O-N emissions among the two experimental years were strongly correlated to temporal difference for soil freezing during winter (80 days with frozen soil and 15 freeze-thaw-cycles vs. 23 days of frozen soil and 2 freeze-thaw-cycles). N_2O-fluxes after spring-ploughing were mainly associated with comparably high soil nitrate contents in the upper soil layer, whereas high N_2O-N losses were inhibited due to low soil water contents during spring. Cultivating maize after spring-ploughing resulted in higher N_2O-N losses (+ 2.35 kg N_2O-N ha^{-1}) compared to grass re-seeding. Higher N-losses could be explained by delayed maize crop establishment in spring and lower N-yields

compared to reseeded grass (184 kg N ha^{-1} vs. 257 kg N ha^{-1}). Slurry application increased N$_2$O-emissions significantly, when grass reseeding occurred in spring.

Table 1. Cumulative N$_2$O-N-emissions (12-months) following ploughing up of grassland in three different resowing treatments. Different capital letters indicate significant differences among treatments. Different lowercase letters shows significant differences among the fertilizer level ($P<0.05$). Mean values are shown ($n\geq3$).

Fertilizer Level	Year	Control	Grassland Renovation (Autumn)	Grassland Renovation (Spring)	Grassland Conversion / maize (Spring)
		Accumulated N$_2$O (kg N$_2$O-N ha^{-1}) 12-months after soil tilling occurred			
0N	1.	0.44Aa	21.31Ca	1.96Ba	4.06Ba
240N	1.	1.40Aa	23.89Ca	3.67Bb	5.70Ca
mean	1.	0.94A	22.6D	2.81B	4.88C
0N	2.	0.59Aa	6.24ABCa	3.90Ba	6.32Ba
240N	2.	0.92Aa	4.89ABCa	5.39Bb	8.24Ca
mean	2.	0.75A	5.56ABC	4.64B	7.28C

Conclusion

If grassland renovation is necessary due to sward deterioration, ploughing and reseeding should be carried out early in the year to reduce the risk of N$_2$O emissions. Slightly higher emissions due to cultivating high yielding maize instead of grass seem to be acceptable, combined with a delayed reseeding following harvest of maize.

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Comparing nitrous oxide emissions from white clover-ryegrass pasture with swards receiving applied synthetic fertilizer

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Abstract

Nitrous oxide (N_2O) contributes 6% of overall global radiative forcing, and has a global warming potential 298 times greater than that of carbon dioxide over a 100-year time period. Agricultural soils are a major source of N_2O, accounting for 35% of annual global emissions. N_2O is produced in soils where the available nitrogen (N) exceeds plant requirements through nitrification, denitrification. To reduce the environmental impact of agriculture, it is imperative to implement mitigation measures that are both effective and deemed practical by farmers. The introduction of clover into grassland swards to fix atmospheric nitrogen and hence reduce the need for inorganic fertilizer inputs (and corresponding N_2O emissions) is one such measure. The aim of this study is to compare N_2O emissions from pasture of differing clover contents, to those of grass-only pastures receiving corresponding N inputs from synthetic fertilizer. N_2O emissions are measured by a closed chamber technique from grass-clover swards with high and low clover contents, and from grass-only swards with high and low synthetic fertilizer input. The research hypothesis is that greater clover content will be conducive to achieving more sustainable grassland-based livestock production systems.

Introduction

Meeting the nutritional needs of an ever-increasing global population will likely create a greater demand for synthetic N fertilizers, consequently increasing N_2O emissions *(Reay et al.*, 2011). By assessing current trends in synthetic fertilizer, a 47% increase in global N_2O emissions from agricultural soils is forecast by 2020 relative to 1990 levels (US EPA, 2006). Legumes offer many attributes which are conducive to environmentally sensitive agriculture. Characterized by their ability to 'fix' atmospheric N, legumes crops are capable of making an important contribution to the future sustainability of grassland systems by displacing dependency on synthetic fertilizers and lowering GHG emissions (Peyraud *et al.*, 2009; Yan *et al.*, 2012).

To reduce agricultural GHG emissions, mitigation measures that are easily implemented are more likely to be adopted by farmers. In a recent study comparing 26 mitigation measures, the inclusion of legumes was deemed as being the most practical to adopt by farmers, while adjudged the most effective in reducing GHGs from grassland-based systems by experts (Jones *et al.*, 2013). Grass-white clover systems are attractive at a farm level, as production levels are not compromised if managed appropriately. With clover dry matter (DM) production of ca. 20 - 30%, grass-white clover swards are likely to yield similar DM contents to that of a perennial ryegrass pasture receiving 150 - 200 kg ha^{-1} yr^{-1} (Andrews *et al.*, 2007), and 70% of a pasture receiving 350-400 kg ha^{-1} yr^{-1} (Andrews *et al.*, 2007; Defra, 2010). Increasing fertilizer costs are likely to make grass-clover systems an attractive alternative in attaining yields (Humphreys *et al.*, 2012). It is estimated that by 2022, the annual UK abatement potential from increased use of legumes could be 0.026 kt N_2O (MacLeod *et al.*, 2010).

Research postulates that N_2O from leguminous residues to be similar to those from the application of synthetic N (Ghosh *et al.*, 2002). The magnitude of emissions, however, is uncertain, with limited research on comparisons of N_2O emissions from fertilizer- and white clover-based systems under similar conditions (Ledgard *et al.*, 2009). Emission intensities are a useful and informative method for comparing emissions between pastures of different compositions (Hansen *et al.*, 2014). This study compares N_2O emissions from white clover-

based grassland systems by analysing swards of differing clover contents to those of grass pastures receiving corresponding N inputs. From this analysis we propose to determine the optimal sward composition in terms of their respective emissions intensities per unit of product.

Methodology

The trial is located in the lowland part of Bangor University's Henfaes Research Station. The experiment is a randomized block design with five treatments and four replicates (Table 1). Each plot measures 25 m^2 in area. The treatments are: 1) a control, 2) a grass-clover sward with 10% clover cover, 3) a grass-clover sward with 50% clover cover, 4) a grass-only sward with an application of 180 kg N/ha (the predicted N-fixation from a sward with 10% clover) (Defra, 2010), 5) a grass-only sward with an application of 300 kg N/ha (predicted N-fixation from a sward with 50% clover) (Defra, 2010). Fertilizer is to be applied in 3 split applications throughout the growing season.

Nitrous oxide (N$_2$O) fluxes are measured by a closed static chamber technique during the 2014 growing season. The chambers are made of polypropylene and fitted into polyethylene collars, which are inserted 5 cm into the soil at least 24 hours before gas samples are taken. Two chambers are assigned to each plot, with additional chambers allocated to a reference plot. Sampling is conducted weekly with an increase in frequency following N application. On each sampling day, flux measurements are conducted between 09:00 and 12:00. N$_2$O emissions from separate urine-treated plots are also measured to simulate urine deposition from sheep fed the biomass from the five treatments described above. Gas samples are analysed using a gas chromatograph (GC) fitted with an electron capture detector (ECD). N$_2$O concentrations at 0, 20, 40, and 60 min are used to estimate N$_2$O flux (g N ha^{-1} d^{-1}) for each chamber. Ancillary soil measurements are also made on each sampling date to get an overall picture of different N forms. N$_2$O emissions are expressed as a function of DM yield, or emissions intensities.

Results and discussion

Both herbage intake, and performance, of livestock is coupled with feed of high clover contents (*Wilkins et al.*, 1994; Ribeiro *et al.*, 2003). Although having advantages in terms of feed characteristics, increased clover content is not correlated to an increase in N$_2$O emissions (Klumpp *et al.*, 2011). The study takes place during the growing season of 2014 and the research hypothesis is that clover content will be conducive in achieving a more sustainable approach to grassland-based livestock production systems.

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Theme 1 posters

Impact of climate change on grassland productivity and forage quality in Austria

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Abstract

Grassland farming is the main land use system in mountainous regions of Austria. Climate change scenarios assume an increase of temperatures of up to 2 °C for the next few decades and also expect reduced rainfall in summer for the alpine space. This change will have an impact on grassland productivity concerning yield and forage quality. To provide grassland farmers and policy makers with relevant information, a series of 27 field experiments was established along a strong gradient of site and climatic conditions in Austria. The sites were clustered into four different climate groups by means of long-term temperature and precipitation values. Multivariate analysis showed that grassland yield and forage quality were mainly affected by the factors year, climate group and management intensity. In 2003 extraordinary weather conditions with high temperatures and below-average rainfall strongly affected grassland productivity concerning dry matter yield but had no significant impact on forage quality. In arid regions considerable yield losses up to 30% occurred, whereas in humid regions yields even increased partly. Our findings clearly demonstrate that adaptation strategies to climate change on grassland have to consider spatial aspects.

Keywords: drought periods, dry matter yield, adaptation strategies, spatial impact

Introduction

Permanent grasslands of different types cover an area of 1.44 million hectares, which is 50% of the total Austrian agricultural area. There are 60,000 grassland farmers, mostly running small- to medium-size enterprises, who focus mainly on the efficient use of farm manure and the production of high quality forage as the most relevant farm internal resources in mountainous regions. Changes in climatic conditions and climate variability, e.g. extreme weather events (heat waves, droughts, *etc*.) are likely to occur more frequently in different spatial and time scales in the future (Eitzinger *et al*., 2009). In order to respond in time it is of great interest for farmers, and also for policy makers, to receive basic information about the regional impact of climate change on grassland yield and forage quality (Meisser *et al*., 2013).

Materials and methods

A multi-site field experiment was established by AREC Raumberg-Gumpenstein on 27 different locations in Austria in the year 2002. The experimental design using three replicates included three cutting intensities (2, 3 and 4 cuts year^{-1}) each with an appropriate level of fertilization (0.9, 1.4 and 2.0 LU ha^{-1}) using slurry or stable manure + liquid slurry respectively, with additional mineral nitrogen fertilizer (50 kg ha^{-1} year^{-1}) for the most intensive variant. The harvesting dates were adapted to the particular site conditions, which varied from 6.4 − 11.1 °C of average yearly temperature, from 548 − 1440 mm of annual precipitation and an altitude from 209 − 1100 m a.s.l. The experimental sites were clustered into four climate groups, based on average temperature and precipitation data. Multivariate statistical analysis were then carried out to identify the most relevant management and site factors which influence dry matter yield and quality parameters.

Results and discussion

Grassland dry matter yield was significantly influenced by the factors year, climate group and management intensity, which explained more than 90% of the observed variation. There was no significant difference between the two humid climate groups which showed higher yields than the sites in arid regions, with the lowest yields occurring under warm conditions (Table 1). A multiple comparison of means showed that in three years (2003, 2007 and 2011) of the total project period, significantly lower yields occurred. In 2003, above-average temperatures were combined with below-average rainfall in almost all parts of Austria and caused dramatic damage and losses in grassland and arable farming. Our analysis (Table 2) showed that there were great spatial differences concerning the impact on grassland yield in this extraordinary year (Schaumberger *et al.*, 2012). Under humid/warm conditions no yield reduction was noticed, and under these humid/warm conditions even an increased yield occurred especially when grassland was cut twice (+10%) or three times (+12%) per year. In contrast, a strong average yield decline of 24% in arid/cold regions, and 29% in arid/warm regions was observed with no significant differences between the three tested cutting/fertilization intensities.

Table 1. Dry matter yield of grassland under different climate conditions in Austria (average of the period 2002-2011; a, b – indicate significant differences between climate groups ($P<0.05$))

Climate groups	humid/warm n=549	humid/cold n=360	arid/warm n=783	arid/cold n=675
DM yield (t ha^{-1} year^{-1})	8.10^a	8.32^a	6.82^b	7.55^c
SD	+/- 2.734	+/- 1.696	+/- 2.723	+/- 2.395

Table 2. Dry matter yield of grassland under different climate conditions in Austria (average of the dry year 2003; a, b – indicate significant differences between climate groups ($P<0.05$)).

Climate groups	humid/warm n=63	humid/cold n=27	arid/warm n=81	arid/cold n=72
DM-yield (t ha^{-1} year^{-1})	8.14^a	8.96^a	4.83^b	5.73^b
SD	+/- 2.457	+/- 0.931	+/- 2.482	+/- 1.816

The crude protein (CP) content was also significantly influenced by the factors year, climate group and management intensity explaining 63% of the variation. On average (2002-2011) the highest CP content was found under humid/warm conditions (121g kg DM^{-1}), the lowest CP concentration occurred in the climate group arid/cold with 115g kg DM^{-1} (Table 3). Within the climate groups, a rising CP content occurred with increasing management intensity (2 cuts > 3 cuts > 4 cuts). In contrast to the decreasing dry matter yield, the CP concentration in the dry year 2003 was significantly higher in all climate groups (ranging from 133 – 146 g kg DM^{-1} with significantly highest values under dry/warm conditions) and also for all tested management intensities (Table 4). These results could also be found for the energy concentration (MJ net energy lactation kg DM^{-1}) in forage that was mainly affected by management intensity (2 cuts > 3 cuts > 4 cuts) but to a lesser extent by weather conditions (Table 3). In 2003 the highest values for energy concentration were measured in cold areas with normally low temperatures, and the lowest energy concentration occurred under humid/warm conditions (Table 4). Even though forage quality was just slightly influenced by the extraordinary weather situation in the year 2003, considerable differences occurred for energy yield and crude protein yield under arid conditions, caused by strong yield decline in these climate groups. To provide the present numbers of livestock on farms with sufficient amount of energy and crude protein additional feedstuff is required to bridge this critical

shortage. Another challenge is to prevent or repair drought damage of the sward by renovation measures using seeds mixtures that are well adapted for dry conditions.

Table 3. Crude protein and energy content of forage under different climate conditions in Austria (average of the period 2002-2011; a, b – indicate significant differences between climate groups ($P<0.05$)).

Climate groups	humid/warm	humid/cold	arid/warm	arid/cold
CP (g kg DM[-1])	115.2[a]	117.7[b]	118.6[bc]	120.8[c]
MJ NEL (kg DM[-1])	4.53[a]	4.79[c]	4.65[b]	4.90[c]

Table 4. Crude protein and energy content of forage under different climate conditions in Austria (average of the dry year 2003; a, b – indicate significant differences between climate groups ($P<0.05$)).

Climate groups	humid/warm n=549	humid/cold n=360	arid/warm n=783	arid/cold n=675
CP (g kg DM[-1])	135.2[a]	134.0[a]	145.7[b]	131.9[a]
MJ NEL (kg DM[-1])	4.49[a]	4.87[b]	4.84[b]	4.92[b]

Conclusion

Our findings clearly indicate that the impact of climate change on grassland productivity in mountainous regions of Austria shows a strong spatial variability and therefore requires different strategies of adaptation. Whereas humid regions with sufficient water supply even benefit from higher temperatures, in arid regions considerable yield losses have to be taken into account. Forage losses can be compensated by the purchase of external feedstuffs for the short term. To counterbalance negative climate change impact on grassland in the long run, there is a need for increased use of seed mixtures which contain drought-tolerant species like lucerne and better-adapted grass and clover cultivars, but the use of irrigation systems also has to be considered seriously.

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Effect of climatic changes on grassland growth, water condition and biomass – the FINEGRASS project

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Abstract

Project FINEGRASS aims at assessing how climate change affects grassland yield production in Poland and North Norway. It also aims to develop tools for monitoring grassland productivity at the national, regional and the individual grassland scale through the use of the newest and most innovative remote sensing and in-situ based methods. Grass biomass from various farmers' fields will be related to satellite image analyses of the same fields to build an explanatory and predictive regression model to assess forage biomass based on grass species, environmental conditions and land management. Satellite images will be processed to create intra-year and inter-year time series of derived indices: Normalized Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI) will be taken from available cloud-free Landsat, ASTER, SPOT, and/or Sentinel-2 satellite imagery.

Keywords: climate change, grassland productivity, remote sensing

Introduction

Climate change influences grassland productivity throughout Europe. The extremes of the weather in winter, often lack of snow cover together with low temperatures, as well as often occurrence of the increased air temperatures early in spring, cause shifts in phenology and disturbance in water balance of the grasslands areas, which influence the grass yield. The lack of precipitation and increase of temperature later in spring and summer also cause reductions in moisture, resulting in changing water conditions in some areas.

Climate change affects grassland yield production in Poland as well as in North Norway. Winterkill is one of the main obstacles for forage production in North Norway. The projected climate change scenarios indicate that the frequency, degree, and length of winter warming events will increase, and may already have increased. These winter warming events can lead to complete snowmelt during winter, thereby increasing the risk for direct frost on plants due to exposure to ambient air and anoxia damage due to accumulation of ground-ice. In Norway, the growing season has been extended by more than one week during the last 30 years (from 1979-2008, compared with 1961-1990) (Karlsen *et al*., 2009). In Poland, the most important abiotic factor limiting grassland productivity is water shortage and distribution during the vegetative season.

There is a lack of tools for efficiently monitoring the effects of climatic trends on grassland productivity on a regional or national level, and therefore there is a need for developing reliable methods for quantifying yields and collecting data on a large scale. Remote-sensing technology may be applied to approach this. Satellite-based radiometers are useful for measuring vegetation characteristics over time across larger areas. Satellite imagery has been used to assess forage production levels over large areas by calculating the Normalized Difference Vegetation Index (NDVI) (Smit *et al*., 2008) or the Enhanced Vegetation Index (EVI)

(Kawamura *et al*., 2005). This technology may also be used to assess the forage grassland production on farm, regional and national levels**.**

The project FINEGRASS entitled 'Effect of climatic changes on grassland growth, its water conditions and biomass' will be conducted for the period of 36 months (Dec. 2013-Dec. 2016). The project aims at the assessment of the influence of climatic changes on grassland productivity as well as development of innovative tools for grassland management on the national, regional and the individual grassland scale. In the FINEGRASS project, the newest and most innovative remote sensing and *in-situ* based methods will be applied as well as interdisciplinary research will be conducted. The project partners consider that this project is an answer to the lack of reliable information about grassland growth conditions and the influence of meteorological conditions, on their status available in the existing databases and official documents, which are being elaborated through traditional methods.

Materials and methods

The overall scope of work as well as the relations between the major research activities within the project are presented on the Figure 1.
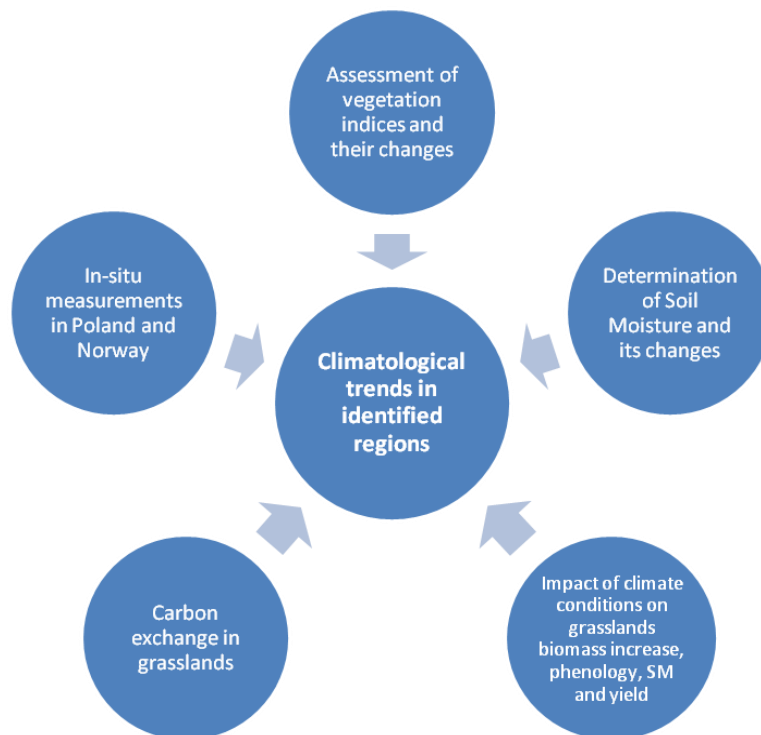
Figure 1. The major project activities and the relations between them

Meteorological data will be gathered from the last 20 years. Years with meteorological anomalies in temperature and precipitation will be noted at each site in Norway and Poland. In North Norway for years with extensive winterkill we will estimate the extent of winterkill using Landsat. To assess if the trend of earlier snowmelt and extended growing season reported in other studies (Karlsen *et al*., 2009) affect grassland productivity, we will use satellite data (MODISand AVHRR for dates prior to MODIS launch) to estimate dates of snowmelt and greening of vegetation for Tromsø. We will compare these data with historical phenological and grass yield data from the Bioforsk Holt research station in Tromsø. The vegetation indices estimated from NOAA/AVHRR data and MODIS data will be analysed in the Poland study sites, and the anomalies of these indices will be connected to the climatological anomalies. Based on satellite-derived surface temperature the method of estimation of heat fluxes applying the energy balance equation will be used (Dąbrowska-Zielińska *et al*., 2009). Ground

measurements from the different managed grasslands (soil moisture, LAI, carbon fluxes and biomass) will be the input into the models that are being derived for the description of grasslands conditions throughout Poland and Norway.

To develop models based on NDVI for estimation of yield and protein content in the sward, we will build regression models to assess how NDVI values over the summer period relate to grass yields (ground truthing, as determined by planned field campaigns, and historical data), including information on how the fields were managed. We will incorporate the use of unmanned aerial vehicle (UAV) as a safeguard against the lack of potential satellite imagery for comparison with fieldwork campaigns. Proximal remote sensing combined with chlorophyll measurements will be used to assess protein content at multiple times in the summer, and correlated with satellite, UAV and NDVI.

Discussion

Climate changes in Poland and Norway may affect the grassland productivity positively and negatively. Higher temperatures with an extended growing season may bring about new opportunities for farmers in the North where forage production is limited by the short growing season. In Poland, this effect allows the possible extension of the grazing season for suckler cows (Goliński *et al*., 2013). Increased precipitation and more unstable winter conditions may also increase the vulnerability of forage production. Extreme winter warming events may lead to de-hardening of grasses or more problems with ice encasement, thus increasing the risk of winterkill (Jørgensen *et al*., 2010). Thus, the projected climate scenarios point in different directions. It is therefore necessary to build new and efficient methods that can be used to monitor the productivity of grasslands to understand trends and anomalies that are likely to continue into the future. This can help in planning for agricultural practices and offsetting financial risks on large scales.

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Generating carbon credits from perennial forage species crops in the Mediterranean region: the case of *Phalaris aquatica* L.

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Abstract

Agricultural practices, like agroforestry, low/no tillage and planting perennial forage crops, have an important potential to increase carbon sequestration as an option for climate change mitigation. The purpose of this study was to quantify the carbon stored in (a) aboveground biomass and, (b) soil organic matter of forage crops, on sites at different altitudes in Northern Greece, planted with the native Mediterranean perennial grass *Phalaris aquatica* L., in order to estimate the potential carbon credits. The results showed that *P. aquatica*, as a perennial forage crop, can provide an alternative agricultural management option for climate change mitigation in the Mediterranean region, due to the large impact on the amount of carbon that can be stored in aboveground biomass and sequestered into the soil.

Keywords: soil organic carbon, biomass, forage species, climate change, carbon offsets.

Introduction

Land use, land-use change and forestry (LULUCF) are a fundamental part of the Kyoto Protocol (KP) (UNFCCC, 1998). Cropland management is an alternative that can be used to achieve the target that each developed country has under the KP (Ovando and Capparos, 2009). In addition, farming systems that increase soil C stocks are fundamental to the sustainability of agricultural production systems. Positive results related to soil C increments, and thus to soil chemical, physical and biological quality, and to mitigation of global warming potential by atmospheric CO_2-C removal, have been obtained by adopting forage farming worldwide (Neal *et al*., 2013). However, the amount of carbon stored in soil and vegetation depends on local site conditions, plant species and land use management. The aim of this research was to quantify the current C stocks aboveground and in the soil of *Phalaris aquatica* L. crops at two sites with different altitudes in Northern Greece, in order to estimate the potential carbon credits generated from this land use.

Materials and methods

Biomass samples of the perennial grass *Phalaris aquatica* L., from rain-fed crops (>10 years old) were collected at the end of the growing season of 2012 and 2013 from two sites in Northern Greece. The sites were at Thermi (40º30′N, 23º4′E) and Chrysopigi (41º10′N, 23º33′E) with different altitudes (30 and 650 m, respectively). Biomass production was measured using 10 (1 x 1 m^2) plots. Harvested biomass was dried at 60 ºC for 48h and milled to 1 mm. The ash-free dry weight (organic matter in biomass) was determined by loss on ignition at 600 ºC for 4h (Allen, 1989). Carbon content was calculated by dividing the percentage ash-free dry weight by 1.8 (Koukoura, 1998). Carbon offsets were estimated after the conversion of 1 t of organic carbon to CO_2 t (by multiplying by 3.67, i.e., 44/12). Soil samples were collected at two depths, 0-15 cm and 15-30 cm, and soil properties were determined using common analysis methods. Total nitrogen and organic matter concentration

were measured by the $K_2Cr_2O_7$ method using the modified Kjeldahl wet digestion procedure of Miller and Keeney (1982). Soil C (t ha^{-1}) was calculated according to the formula:

C (t ha^{-1})= [C (%) x soil depth (m) x 10000 x bulk density (g cm^{-3}) x 10]/1000 (Alifragis, 2008). One-way Analysis of Variance was used to compare means in two sites. Further differences were evaluated with the LSD post hoc test, at P=0.05 (Kinnear and Gray, 2008).

Results and discussion

Mean organic matter concentration (kg t^{-1} DM) in aboveground biomass of *P. aquatica* crops was significantly higher at Chrysopigi than the Thermi site (Table 1). This difference could be attributed to different soil types: at the Chrysopigi site the soil is characterized as sandy loam, while at the Thermi site it is clay loam (Table 2). According to Burval (1997), biomass of the species *P. arundinacea* grown on high clay content soils had a high amount of ash. Furthermore, there were higher mean carbon stocks (t ha^{-1} DM) stored in the aboveground biomass of lowland crops than in upland crops, mainly due to significant yield differences (Pappas *et al.*, 2012) rather than differences in the organic carbon concentration (kg t^{-1} DM), which correspond to higher mean carbon offsets (11.37 t CO_2 ha^{-1}).

Table 1. Mean values (with s.d.) of ash, organic matter, organic carbon, biomass yield, organic carbon and CO_2 (t ha^{-1}) stored in aboveground biomass of *Phalaris aquatica* L. crops grown over 2 years on sites at different altitudes.

Site	Chrysopigi			Thermi		
Year	2012	2013	Mean	2012	2013	Mean
Ash (g kg^{-1} DM)	64 (1.1)	87 (0.6)	76 (13)	117 (0.7)	98 (0.6)	107 (11)
Organic matter (kg t^{-1} DM)	936 (1)	913 (0.5)	924 (13.3)	883 (0.6)	902 (0.7)	893 (11)
C (kg t^{-1} DM)	520 (0.6)	507 (0.8)	514 (7.4)	491(0.4)	501 (0.6)	496 (6.1)
Biomass yield (t ha^{-1} DM)	4.9 (0.2)	4.9 (0.3)	4.9 (0.5)	6.2(0.5)	6.3(0.4)	6.25(0.3)
C (t ha^{-1} DM)	2.55 (0.6)	2.49 (0.8)	2.52(7.4)	3.04 (0.2)	3.16 (0.3)	3.10 (0.6)
CO_2 (t ha^{-1} DM)	9.35(0.7)	9.12 (0.3)	9.24(1.3)	11.17(0.5)	11.59 (0.3)	11.37 (0.2)

Table 2. Mean of soil organic matter, organic carbon, organic carbon and CO_2 (t ha^{-1}) sequestered in *Phalaris aquatica* L. crops grown on sites at different altitudes.

Site	Chrysopigi (650 m)		Thermi (30 m)	
Depth (cm)	0-15	15-30	0-15	15-30
Soil type	Sandy Loam	Sandy Loam	Clay Loam	Clay Loam
Bulk density (g cm^{-3})	1.31 (0.17)	1.0 (0.08)	1.40 (0.15)	1.0 (0.12)
C:N	11.6	10.3	11.7	10.8
C (%)	1.15 (0.1)	0.93 (0.05)	0.82 (0.08)	0.65 (0.07)
C (t ha^{-1})	22.9 (1.98)	14 (0.69)	17.1 (1.85)	9.7 (1.06)
CO_2 (t ha^{-1})	84.0(7.25)	51.4 (2.62)	62.8 (6.92)	35.7 (3.90)

High quantities of total organic carbon (t ha^{-1}) were sequestered in the soil at 0-30 cm depth at both sites (Table 2). The C content was higher in the topsoil (0-15 cm) than in the 15-30 cm depth. The same trend has been reported by several studies evaluating carbon sequestration under different perennial grasses cultivated on agricultural land (Omonode and Vyn, 2006; Clifton-Brown *et al.*, 2007; Christensen *et al.*, 2009). According to Zan *et al.*, (2001) the conversion of agricultural land to perennial forage crops can be expected to increase C stored in above- and belowground biomass and in the soil organic matter because of their perennial nature and greater root biomass production. Total soil carbon sequestration (in t ha^{-1}) was significant higher in the upland (36.9 t ha^{-1}) than the lowland (26.8 t ha^{-1}) crop, corresponding to higher carbon offsets (tonnes of CO_2) per hectare (135.4 and 98.5 t ha^{-1} respectively). This was due to higher C content in both depths, indicating differences in litter decomposition rates between the two sites. Moreover, carbon sequestered in the aboveground biomass, and

especially in the soil, is stable for a long period of time and fulfils the criteria of additionality, permanence, leakage and double counting in order to be verified as carbon credits in voluntary carbon markets (Murril, 2008; Papaspyropoulos *et al*., 2013).

Conclusion

The growing of perennial C_3 forage crops is a potential climate-change mitigation activity in the Mediterranean region, as these crops can store and sequester larger amounts of carbon in the aboveground biomass and into agricultural soils, as shown in the case of Northern Greece. These carbon offsets can be efficiently introduced as carbon credits in evolved carbon markets.

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Agroforestry systems: an option for mitigation and adaptation to overcome global climate change

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Abstract

Agroforestry, a combination of a woody (shrub/tree) with an herbaceous component (crops/pasture) is considered an important tool to mitigate and adapt agrarian systems to global climate change. This fact is based on the capacity that AGF systems have to preserve C already accumulated in the woody component but also to increase C sequestration in a tree-less system when trees are planted. Nitrate and CO_2 emissions can be reduced by the consumption of the biomass of the understory in forests with high fire risk, but also for the better use of fertilizers in more open systems that will contribute to mitigate the negative effects of this fertiliser inputs on GHG atmosphere release. Resilience is also improved as biodiversity in AGF systems is usually higher than in tree-less systems.

Keywords: biodiversity, GHG, carbon sequestration, land use, land management

Introduction

Agroforestry (AGF) systems include sustainable land management practices that comprise at least two components, one woody (tree /shrub) and one herbaceous (grass /crop including forage), but may also involve livestock as a third component. Man is part of the AGF systems as he manages this semi-natural system attempting to enhance synergies between the different components, in order to achieve optimal use of resources such as light, water and nutrients. When establishing an AGF system, the main ecological factor to consider is the amount of the radiation that can reach the lower strata of the AGF system. Therefore, when an AGF system is established in areas without initial woody cover, we should consider aspects such as the density and distribution of the woody component. If the AGF systems are set on systems with an existing woody component, we focus on optimizing the density of the woody component to enhance the overall productivity (Mosquera-Losada *et al.,* 2009).

The use of AGF systems and relationship to reducing the effects of climate change are clear. The Kyoto Protocol highlights that activities related to change of use (reforestation deforestation (Art 3.3) and management of forest lands, agricultural, livestock and revegetation (Art 3.4), can be used to mitigate and reduce emissions of greenhouse gases (GHG) (UN, 1998). Burley *et al.* (2007) indicate that forest land management can reduce the effect of the emission of GHG through carbon (C) sequestration and through the promotion of reduced emissions from vegetation (reducing fire hazards, deforestation). European countries used these mechanisms (items 3.3., and 3.4) to meet the Kyoto targets (EEA 2009). AGF systems are currently included in Canada and USA, and in European policies to make traditional-recent systems (monocrops) more sustainable (Schoeneberger *et al*., 2012). Studies with aims of comparing AGF systems or exclusively agricultural land use reflected a clear advantage to the former (Nair *et al*., 2009; Holwett *et al*., 2011; Mosquera *et al.,* 2011).

Preservation and maintenance of already accumulated carbon

AGF systems can contribute to preserve and maintain C stored in terrestrial ecosystems as they can avoid 1) complete deforestation of forestlands to grass or forage lands, and 2) they reduce forest fires. Total deforestation is one of the most important pathways that contribute negatively to the net GHG emissions, mainly because: (i) it involves the release of C stored in the tree

itself and from the soil and (ii) deforested area stops photosynthesis, thus C fixation is reduced. If AGF system practices (instead of complete deforestation) is established, a high volume of soil could be occupied by tree roots in lower soil horizons than roots of herbaceous crops, even very far away from the tree canopy; e.g. Spanish dehesa with < 50-75 trees /ha can have roots over 50 m from the tree trunk below the rhizosphere of the crop (Howlett *et al*., 2012; Mosquera-Losada *et al*., 2012). This is very important because roots are one of the main sources of soil carbon. Soil C represents >65% of the C sequestered in terrestrial ecosystems. Moreover, partial deforestation to increase forage/pasture growth can contribute to the conservation and maintenance of the C accumulated compared to systems that promote total deforestation C, without causing a significant reduction of crop production. For example, tree cover close to 55% results in a decline in crop yields around 50% in temperate conditions (Mosquera-Losada *et al*., 2009). Further, the use of machinery in AGF systems can be performed with low tree density or with tree distribution in groups or hedges. Implementation of silvopastoral systems reduces fire risk through the direct understory consumption, reducing the existing biomass (load fuel) so avoiding potential C emissions atmosphere.

Carbon sequestration promotion

Land use change

The increase of C in a system caused by the introduction of a tree species in a treeless field is associated with the rate of tree growth (mainly linked to root development at deeper soil layers) and the effect it causes on the understory. It has to be taken into account also that tree species with high growth rates (e.g. poplar) sequester more C per year than those with low growth rates, but the number of years for tree harvest is lower in the fast-growing tree species and therefore the potential of GHG release from the stand and soil is earlier. Promotion of AGF systems instead of simpler (monocrop) systems is usually linked to high levels of biodiversity (alpha biodiversity) because of the spatial heterogeneity caused by the tree (shadow), and the grazing animals (trampling, selective grazing, irregular faeces distribution) at plot level, but also preserves and increase biodiversity (beta biodiversity) at a landscape level in those transhumant systems where woody component is important. It is noteworthy that the promotion and preservation of species biodiversity play a key role to promote adaptation of ecosystems to the impacts of climate change and therefore its resilience.

Management

Land management can also be a source of GHG. For example, tillage and ploughing as well as fertilization are usually associated with a release of GHG caused by soil mineralization or the inefficient use of fertilizers. However, woody components of AGF systems can absorb the nutrients released by decomposition after ploughing (different N forms) or after fertilizer applications. Modern AGF systems established with Juglans at low density have shown that grazing improves the amount of C sequestered in the soil, when compared with tillage for understory control. C decrease in ploughed areas is probably associated with the lack of vegetation during several months per year.

Replacement of materials and fossil fuels

One of the main reasons for the increasing atmospheric GHG concentrations is the use of fossil fuels releasing C stored over millions of years. That is why the use of biomass (from AGF activities such as pruning, clearing, thinning, logging) is promoted as a renewable energy source. Such uses may also be undertaken as part of AGF systems, and have a great tradition in the Spanish dehesas, where in order to increase fruit production and shaping the trees, pruning is performed and generates income from land (charcoal). Today, the existence of pruning tools with hydraulic sytems and rising platforms could facilitate this type of use

because they reduce the costs of labour, a major limiting factor for the use of biomass in developed countries as an energy source.

In recent years the use of woody crops for biomass production for energy purposes has been encouraged in different countries of Europe. These systems used woody crops at high densities (alder, eucalyptus, poplar and acacia). Afterwards, mechanized harvesting is performed 3-6 years after development depending on the site quality, and regrowth for a new crop is allowed without cultivation. In Germany, these tree species have been combined with crops (herbaceous species). This type of AGF, called 'alley cropping,' uses strips 10 m wide of woody crop in combination with other strips of 15-20 m of arable crop (Mosquera *et al.*, 2011). Woody species interspersed with crops in alley cropping systems have been shown to contribute significantly to increased C sequestration, and to biodiversity in the soil, compared to crop areas without woody species (Quinkenstein *et al.*, 2009; Matos *et al.*, 2011).

Conclusions

AGF systems are a land management strategy, which compared with others, may allow improved resilience of agro-ecosystems to climate change impacts. This improvement in the response to climate change is based on their capacity to preserve and maintain the C accumulated, increase the C sequestered and provide materials and fuels to replace fossil fuels. This greater adaptation capacity is mainly based on improvement of biodiversity and better use of resources (nutrients, light and water) compared with other land management.

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Drought tolerance of the *Lolium multiflorum-Festuca arundinacea* introgression forms

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Abstract

Italian ryegrass (*Lolium multiflorum* Lam.) has high forage quality but low tolerance to abiotic stresses. Tall fescue (*Festuca arundinacea* Schreb.) expresses higher tolerance, especially to drought conditions. The hybrids of both species and their introgression derivatives combine the complementary attributes of *L. multiflorum* and *F. arundinacea*. The main aim of this study was to evaluate the persistence of BC_4 *L. multiflorum-F. arundinacea* introgression forms after long-term drought in simulated field conditions and their ability to recover after stress termination. Diploid and tetraploid introgression forms, together with *Lolium* controls, were subjected to tests for drought tolerance under 'rain-out' shelters in the field. Fresh matter yield after 14 weeks of drought and re-growth after two weeks of further re-watering for each genotype and average values of these parameters for the whole populations, were estimated. The population of diploid introgression forms demonstrated higher level of drought tolerance, compared to the population of diploid *L. multiflorum*. However, among the best diploid *Lolium* controls, genotypes with relatively high drought tolerance also existed, showing even higher values of the analysed parameters compared to the best BC_4 forms. The populations of tetraploid introgression forms and controls demonstrated higher average fresh matter yield and re-growth, compared to the diploids.

Keywords: *Lolium multiflorum*, *Festuca arundinacea*, drought tolerance, introgression

Introduction

Italian ryegrass (*Lolium multiflorum* Lam.) has relatively high forage quality but rather poor tolerance to abiotic stresses (Humphreys and Thomas, 1993). Conversely, tall fescue (*Festuca arundinacea* Schreb.) is characterized by high level of tolerance, especially to drought, but it cannot compete with *Lolium* in terms of productivity and quality in favourable environmental conditions (Kosmala *et al*., 2007, 2012). These two species can be hybridized, and their homoeologous chromosomes pair and recombine frequently in the intergeneric hybrids. Thus, it is possible to combine their complementary traits within a single genotype on the way of crossing (Humphreys and Pašakinskienė, 1996; Kosmala *et al*., 2007; Perlikowski *et al*., 2013). The current research is a part of the project focused on development of new introgression *Lolium* cultivars with improved tolerance to water deficit. The main aim of this study was to evaluate the persistence of *L. multiflorum-F. arundinacea* introgression forms after long-term drought in simulated field conditions and their ability to recover after stress termination.

Materials and methods

The intergeneric partially fertile pentaploid ($2n = 5x = 35$) *L. multiflorum* × *F. arundinacea* hybrid was produced by crossing autotetraploid ($2n = 4x = 28$) *L. multiflorum* with hexaploid ($2n = 6x = 42$) *F. arundinacea*. The hybrid was then backcrossed to *L. multiflorum* cultivars – diploid cv. Tur and tetraploid cv. Lotos. After three subsequent backcrosses of the backcross (BC) plants to diploid or tetraploid *Lolium*, the introgression BC_4 populations were generated. After initial establishment of 100 diploid and tetraploid BC_4 plants in the glasshouse, 35

randomly selected genotypes from each group were subjected to tests for drought tolerance. Simultaneously, 35 genotypes of *L. multflorum* cv. Tur and 35 genotypes of cv. Lotos, were used as controls. The tests were performed using simulated conditions under 'rain-out' shelters at Danko Plant Breeding Ltd., in Szelejewo, Poland. Each shelter was covered with foil protecting it against rainfall and was equipped with soil draining system. In May 2011, three clones of each tested genotype were planted randomly under three distinct rain-out shelters ('dry' shelters), and one clone was planted in the field with no protection against rainfall, as a control ('irrigated' shelter). During the experiment (14 weeks of drought followed by two weeks of re-watering; June – October 2011), soil humidity under the shelters was monitored. Three harvests were taken during drought period (after four, 10 and 14 weeks of drought) at a cutting height of 8 cm. Fresh matter (FM) yield after 14 weeks of drought and re-growth (RG; scale 0 – 9) after two weeks of re-watering were estimated. Fresh matter yield of each genotype after drought conditions, as well as RG after re-watering, were expressed as a mean score (arithmetic mean of three scores derived from three clones from three distinct 'dry' shelters). Differences in FM yield and RG between the genotypes were evaluated using Tukey's HSD tests. Moreover, for each analysed parameter also an average score for the whole population was calculated, involving all the genotypes of this population under 'dry' shelters. The measured parameters were treated as the indicators of drought tolerance for particular genotypes and populations. The results of plant selection with reference to drought tolerance of tetraploid introgression forms have been published already. Some selected tetraploid genotypes were used in more detailed physiological and molecular research to find the cell components crucial for tolerance development (Perlikowski *et al*., 2013). Herein, the results obtained for diploid genotypes (introgression forms and controls), are shown and discussed.

Results and discussion

The level of soil humidity under the 'dry' shelters and the 'irrigated' one on the day of drought initiation was similar. It decreased continuously under the dry' shelters as drought conditions progressed, and after 14 weeks of drought it was lower, compared to the level revealed for the 'irrigated' shelter. Two weeks after re-watering initiation, soil humidity under 'dry' shelters increased to the level observed under the 'irrigated' shelter (data shown in Perlikowski *et. al*., 2013). The average FM yield per genotype in the population of diploid introgression forms after 14 weeks of drought reached the value of 14.8 g and it was higher, compared to the value calculated for the control population of *L. multiflorum* cv. Tur (10.6 g). However, a big variation with respect to that trait was also observed among the particular genotypes within each analysed group (the introgression forms and *Lolium* controls ranged from 1.5 to 35.3 g and from 0.0 to 42.8 g, respectively). Generally, this tendency was also observed for the RG parameter. After two weeks of re-watering, the average RG reached value of 3.9 (range: 1.6 – 6.7) and 2.9 (range: 0.3 – 8.3) per genotype for the introgression forms and *Lolium* controls, respectively. The values of FM yield and RG for the clone present under 'irrigated' shelter were always higher, compared to the average values calculated for the corresponding population under 'dry' shelters. As demonstrated earlier (Perlikowski *et al*., 2013), the populations of tetraploid introgression forms and *L. multiflorum* cv. Lotos were shown to be better with respect to the average FM yield after 14 weeks of drought, and the average RG parameter after two weeks of re-watering, compared to the diploids. The average FM yield reached the value of 31.7 g and 19.9 g, and re-growth – 5.2 and 4.0 per genotype for the population of tetraploid introgression forms and controls, respectively. The comparisons with relation to the RG parameter among the best genotypes selected within the populations of diploid and tetraploid introgression forms and controls are shown in Figure 1.
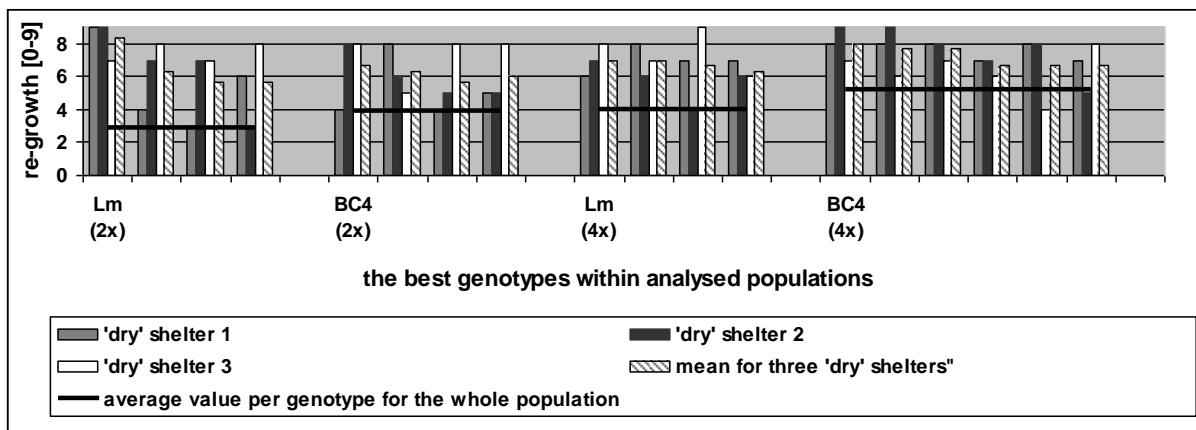
Figure 1. Re-growth after two weeks of re-watering (scale 0 – 9) of the selected diploid and tetraploid BC$_4$ *L. multiflorum-F. arundinacea* introgression forms and *L. multiflorum* (Lm) controls.

Conclusions

The analysed population of diploid *L. multiflorum-F. arundinacea* introgression forms demonstrated higher level of drought tolerance, compared to the population of diploid *L. multiflorum* cv. Tur, manifested by higher average FM yield after 14 weeks of drought and higher average RG after re-watering per genotype. However, among the best diploid *Lolium* controls, genotypes with relatively high drought tolerance also existed, showing even higher values of the analysed parameters, compared to the best BC$_4$ forms. As had been expected, the populations of tetraploid introgression forms and controls demonstrated higher average FM yield and RG, compared to the diploids.

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Effect of water stress on *Lotus corniculatus* L. nutritive value at different stages of maturity

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Abstract

The aim of this study was to estimate the effect of moderate water stress on the nutritive value of *Lotus corniculatus* L. at three phenological stages. Plants of a natural population from Drama, Greece were tested under two irrigation levels: 1) up to field capacity, and 2) 40% of field capacity. The plants were harvested at three phenological stages: early vegetative, flowering and start of fruit formation. Samples were analysed for Crude Protein (CP), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL). Dry matter digestibility (DMD) was calculated. Moderate water stress led to decreased fibre concentration and increased the digestibility. It reduced CP content only at the early vegetative stage. It can be concluded that moderate water stress had slightly affected the nutritive value of *Lotus corniculatus* and probably in a positive way.

Keywords: legumes, drought, phenological stages, feed quality

Introduction

Adequate water supply is a crucial factor for grassland forage production (Hopkins and Del Prado, 2007). It is well known that forage legumes differ in drought-stress sensitivity (Dierschke and Briemle, 2002) and limited water supply can have strong effects on their production (Foulds, 1978). However, knowledge about the influence of water stress on the nutritive value of legumes is limited and inconsistent (Kuchenmeister *et al.*, 2013). *Lotus corniculatus* L. is a promising drought-resistant forage legume of high nutritive value (Escaray *et al.*, 2012). Additionally, condensed tannins present in its leaves prevent bloating in ruminants and protect plant proteins in the rumen from degradation (Waghorn *et al.*, l987). Its overall forage quality under drought conditions is better than that of *Medicago sativa* due to higher leaf:stem ratio, and delayed maturity (Peterson *et al.*, l992). The aim of the present study was to investigate the effect of water stress to the nutritive value of *L. corniculatus* in different phenological stages.

Materials and methods

Plants of a natural population of *L. corniculatus* were collected from Drama, northern Greece, at various elevations (from 100-500 m) in September 2012. Thereafter, they were transplanted into plastic pots filled with organic matter at the farm of the Aristotle University of Thessaloniki, northern Greece, altitude 10 m asl. The climate of the area could be characterized as Mediterranean semiarid (mean annual precipitation 443 mm; mean annual temperature 15.5° C). For this experiment, 32 uniform plants were selected and transplanted into pots (diameter 16 cm, height 45 cm), filled with medium-texture soil and placed under a transparent shelter in spring 2013. Drip irrigation was applied in two levels: 1) up to field capacity (W), and 2) 40% of field capacity (WL). Pots were placed in a completely randomized design. The plants were harvested three times at 4 cm above the soil surface, at the following phenological stages: 1st

cutting: 21 May (early vegetative), 2nd cutting: 5 June (flowering) and 3th cutting: 28 June (start of fruit formation). Aboveground biomass from every individual plant was oven-dried at 60° C for 48 h, ground through a 1 mm screen and analysed for Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL) (Van Soest *et al*., 1991) using the ANKOM fibre analyser (ANKOM Technology Corporation, Macedon, NY, USA). Nitrogen was determined using the Kjeldahl procedure (AOAC, 1990), and crude protein was calculated as N content × 6.25. Dry matter digestibility (DMD) was calculated using the equation proposed by Oddy *et al*. (1983): DMD %=83.58-0.824 ADF% +2.626 N%. Two-way ANOVA of the data was performed using SPSS® statistical software v. 18.0 (SPSS Inc., Chicago, IL, USA), in order to determine differences among the phenological stages and water treatments. The LSD at the 0.05 probability level was used to detect the differences among means (Steel and Torrie, 1980).

Results and discussion

NDF, ADF and ADL (across phenological stages) of limited watered plants were significantly reduced and, as a consequence, the DMD was significantly increased, while the CP was not significantly affected (Table 1). ADF (across water-stress treatment) was significantly increased from the early vegetative to the fruit formation stage, while the opposite trend was recorded for CP and DMD (Table 1).

Table 1. Chemical composition (g kg^{-1} DM) and DMD (%) of *Lotus corniculatus* samples in two irrigation levels and three phenological stages

Water levels	CP	NDF	ADF	ADL	DMD
W	182.5a*	423.8a	286.6a	90.1a	68.5b
WL	178.4a	385.8b	254.1b	83.6b	70.1a
Phenological stage					
Early vegetative	208.8a	396.1a	259.7b	85.9a	71.1a
Flowering	169.9b	414.8a	292.8a	89.2a	69.3b
Fruit formation	162.6b	403.4a	291.6a	85.4a	67.4c

* Different letters in each column within irrigation level or phenological stage indicate significance at $P \leq 0.05$

NDF and ADL were not affected by the vegetative stage. Significant interaction was observed between the water treatment and the phenological stages only for CP (Figure 1). CP content was significantly higher in the well-watered plants only during the early vegetative stage.
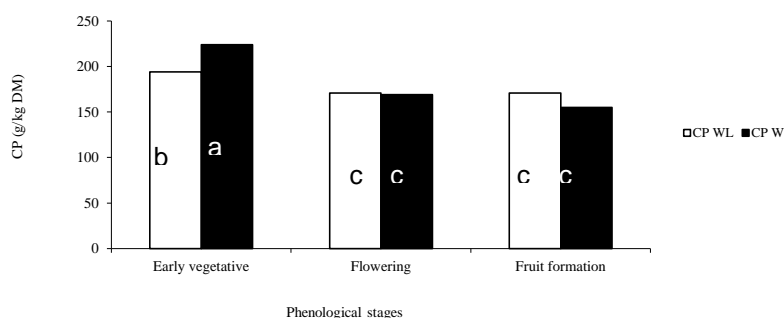
Figure 1. Crude protein (g kg^{-1} DM) of *Lotus corniculatus* samples at three phenological stages and two irrigation levels: W (up to field capacity) and WL (40% of field capacity)

Similar to our results, Kuchenmeister *et al*. (2013) reported that moderate water stress reduced NDF and ADF, while it had no severe effects on CP content. Many interacting factors including the stage of plant development, leaf:stem ratio, environmental conditions or availability of nutrients could affect fibre concentration (Buxton, 1996, Fulkerson *et al*., 2007). The availability of N depends on N fixation but the concentration in the plant also depends on the

amount of biomass production. Kuchenmeister *et al*. (2013) explained the reduced CP concentration under strong water stress to reduced N fixation. It seems that moderate stress did not affect N fixation. However, the lower CP content of water-stressed plants at the early vegetative stage could be associated with higher biomass production at this phenological stage.

Conclusion

Moderate water stress led to decrease fiber concentration and increase the digestibility. Moreover, it reduced CP content only in early vegetative stage. It can be concluded that moderate water stress had slightly affected the nutritive value of *Lotus corniculatus* probably in a positive way.

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Impact of limited irrigation on water economy and photosynthetic performance of *Lotus corniculatus*

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Abstract

Spatial and temporal aspect of drought phenomena in Mediterranean areas force plants to grow under conditions of water deficit. Resistance of a plant species to drought is usually expressed by higher water-use efficiency and maintenance of productivity at high levels. The aim of this study was to evaluate the physiological responses of *Lotus corniculatus* under water-stress conditions. Plants from a natural population of a semi-arid area of northern Greece (Drama) were selected and transplanted to pots. After a period of plant adjustment, two irrigation regimes were used: a) irrigation up to field capacity, and b) partial irrigation in order to maintain water deficit conditions in the soil. Measurements of water potential, assimilation rate, transpiration rate, stomatal conductance and chlorophyll fluorescence were conducted at four growth stages. Water-use efficiency was estimated as the ratio of assimilation to transpiration rate. Our results showed that limited irrigation of *L. corniculatus* did not significantly affect the photosynthetic and photochemical performance, as well as the water-use efficiency. Therefore, *L. corniculatus* could be a suitable and valuable candidate for improving grassland vegetation in dry areas.

Keywords: photosynthesis, stomatal conductance, water use efficiency, *Lotus corniculatus*

Introduction

Drought is a common phenomenon in Mediterranean areas, especially during summer, and has many adverse impacts on plants, inhibiting their growth (Asgharipour and Heidari, 2011). The effect of drought on growth and yield of a species depends upon the severity, duration and timing of water deficit. The degree of plant response to water stress varies among species. Plants develop morphological and physiological adaptation mechanisms, such as reduction of leaf area, stomatal closure and regulation of water potential, in order to minimize water loss and, therefore, to survive during periods of water stress (Passioura, 1997; Sarker *et al*., 2004; Karatassiou *et al*., 2009). Understanding these mechanisms and controlling water economy and plant resistance to drought could increase food productivity and quality. Available knowledge on the effect of drought stress on *Lotus corniculatus* is limited. *Lotus corniculatus* is a worldwide-distributed species that grows under a wide range of environmental conditions and can be found in different microclimates of northern Greece. It is considered to be of high palatability, suitable for vegetation improvement of grasslands, and is usually used for ecological restorations of soils affected by nutrient deficiency, salinity, drought, or contaminants (Escaray *et al*., 2012). The aim of the research reported in this paper was to determine the physiological responses of *L. corniculatus* under conditions of water deficit.

Materials and methods

Lotus corniculatus plants were collected from three different locations of Drama (TEI-labor houses, Horisti, Lydia) northern Greece in September 2012 and transplanted into small pots.

In March 2013, 32 plants were transferred to larger pots (16 cm diameter and 45 cm height), filled with soil of medium texture. The pots were placed under a transparent shelter in the experimental area. Microclimatic conditions of the experimental area are shown in Table 1. Drip irrigation was applied at two levels: full irrigation up to field capacity (FI) and limited irrigation (40% of FI) (LI). We followed a completely randomized experimental design with four replicates. Measurements were performed during spring 2013 on four different dates corresponding to four growth stages: early vegetative, vegetative, flowering and start of fruit formation. The measurements were carried out between 9.30 and 12.00. Water potential (Ψ) of the upper part of the stem was measured, using a pressure chamber (SKPM 1400, Skye Instruments Ltd, Llandrindod Wells, UK) as the leaf of *L. corniculatus* is too small to be measured. Assimilation rate (A), stomatal conductance (Gs) and transpiration rate (E) were measured with a portable photosynthesis system (LCpro-SD, ADC Bioscientific Ltd, Hoddesdon, UK) on the abaxial leaf surface. Water-use efficiency (WUE) was estimated as the ratio of assimilation to transpiration rate. The ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) was measured at dark-adapted for 20 min leaves, using a chlorophyll fluorometer (OS 30p+, OptiSciences Inc, Hudson, USA). Statistical analysis was performed using the SPSS statistical package (SPSS for Windows, standard version, release 17.0; SPSS, Inc., Chicago, USA). Analysis of variance (ANOVA) was used to compare the irrigation treatments throughout the growing season and at each plant growth stage. A significance level of 5% was used throughout.

Results and discussion

Among the physiological parameters tested, irrigation treatment significantly affected only the *L. corniculatus* water potential. Throughout the growing season, *L. corniculatus* plants under limited irrigation had significantly lower water potential (-1.18 MPa) (Table 1) compared to plants under full irrigation (-0.59 MPa). Plants under full irrigation exhibited higher but not significantly different values of assimilation rate, stomatal conductance, transpiration rate, WUE and Fv/Fm compared to plants under limited irrigation (Table 1).

Table 1. Microclimatic conditions of the experimental area during the growing period.

Dates	Temperature (ºC)	RH (%)	PPFD (μmol m^{-2}s^{-1})	VPD (KPa)
1	32.6	13.8	872	4.24
2	27.7	19.7	941	2.98
3	24.3	33.5	1304	2.02
4	30.6	38.5	822	2.70

Assimilation rate, stomatal conductance and WUE varied significantly during the different growth stages, being lower at the end of the growing season (data not shown), probably corresponding to the altered needs of the plant at each growth stage and/or to the environmental conditions. In addition, water potential was maintained at high levels until the middle of the growing season, sharply decreasing towards the end. In contrast, the transpiration rate and the chlorophyll fluorescence ratio Fv/Fm remained unchanged throughout the various growth stages. Our results showed that limited irrigation had rather a small effect on the photosynthetic mechanism of *L. corniculatus*, because the plants maintained their photochemical efficiency and their stomatal apparatus open, and therefore they continued to photosynthesize despite the low water-potential values. Nowadays, it is recognized that some plants are adapted to photosynthesize even under low water potential, using mechanisms that tend to maintain turgor, producing and conserving osmolytes in order to protect tissues from dehydration (Berkowitz, 1998; Jones, 2004).

Conclusion

Limited irrigation did not significantly affect the photosynthetic and photochemical performance, as well as the water use efficiency, of *L. corniculatus* plants. Therefore, *L. corniculatus* could be considered as a suitable and valuable candidate for improving grassland vegetation in dry areas or for water-saving cultivation.

Table 2. *Lotus corniculatus* physiological responses under two different irrigation treatments throughout the growing season (n=16).

Treatment	Physiological parameters					
	A (μmol m^{-2}s^{-1})	E (mmol m^{-2}s^{-1})	Gs (mol m^{-2}s^{-1})	Ψ (MPa)	WUE	Fv/Fm
Full Irrigation	4.70±0.78	1.71±0.30	0.06±0.01	-0.59±0.23	3.06±0.50	0.81±0.01
Limited Irrigation	3.81±0.71	1.49±0.13	0.04±0.01	-1.18±0.33	2.43±0.37	0.79±0.01
Significance	ns	ns	ns	*	ns	ns

Significance: * $P<0.05$; ns, not significant.

Acknowledgments

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Drought resistance of selected forage legumes for smallholder farmers in East Africa

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Abstract

To improve feed availability for smallholder farmers in East Africa, we investigated the biomass production and drought resistance of five forage legumes in semi-arid environments of Rwanda and Uganda. The crops were grown under rain-fed conditions (control) and with additional irrigation (irrigated) and harvested at two-monthly intervals four to five times. Before harvests, the youngest leaves of the crops were sampled for stable carbon isotope analysis for an indication of intrinsic water-use efficiency. This is the first time carbon isotope data are reported for these species. The total annual dry matter production was larger for the legumes grown in Uganda than in Rwanda. However, there were large differences among harvests, with the variability between smallest and largest harvests being similar for both countries. Carbon isotopic signatures were more enriched for samples from Rwanda, hinting at a larger intrinsic water-use efficiency under the local conditions. *Canavalia brasiliensis* had most enriched carbon signatures in both countries, coupled with acceptable biomass production, and should be further investigated for adaptation in smallholder farming systems.

Keywords: yield, stable isotopes, $\delta^{13}C$, irrigation, intrinsic water-use efficiency, forage

Introduction

Having access to reliable forage of sufficient quality, especially during the dry season, still poses the main challenge to smallholder dairy producers in semi-arid areas of East Africa (Hall *et al*., 2007). Typically, the amount of milk gained as well as the length of the reproductive cycle of the animals depends on the amount and quality of forage available. To overcome the difficulties, zero-grazing systems have been developed. In contrast to most other crops, many forage species can also be grown on marginal lands and thus provide an opportunity for farmers to build a livelihood. Legumes can offer the extra benefit of improving the nitrogen-poor soils. However, drought-resistance is an important key trait of the plants to be used successfully. Therefore, in this study, five forage legumes were tested for their ability to provide biomass in field trials in Uganda and Rwanda. Measurements of the stable carbon isotopes ($\delta^{13}C$ signatures) were used to assess the drought resistance of the legumes. The ^{13}C signatures are directly proportional to the intrinsic water-use efficiency, i.e. the amount of carbon assimilation per stomatal conductance for water (Farquhar *et al*., 1989). We hypothesized that there would be differences among the legumes in biomass production during wet and dry conditions and in intrinsic water-use efficiency.

Materials and methods

Five forage legumes, namely *Lablab purpureus*, *Desmodium uncinatum* cv. Silver leaf, *Desmanthus virgatus*, *Macroptilium bracteatum* cv. Burgundy bean, and *Canavalia brasiliensis*, were grown in a completely randomized block design (plots of 3 m x 6 m, 1 m in between plots) with five replicates, with or without additional irrigation (by hand, if no

precipitation had fallen the previous day) in field sites in Uganda (National Livestock Resources Research Institute, Tororo district; annual rainfall 1130-1720 mm, AATF, 2009) and Rwanda (Rwanda Agriculture Board, Karama Research Station, Bugesera district; annual rainfall 845 mm, REMA, 2007). Planting was done in the rainy season (October 2012) at recommended rates and spacing. Four (Rwanda) or five (Uganda) harvests took place at two-monthly intervals until June 2013. Biomass of 1 m² was harvested 10 cm above the ground, and samples of about 200 g were oven-dried (60 °C for 48 hours) and weighed. Just before harvest, the youngest leaf of several plants was sampled for stable isotope analysis. Isotope measurements were done on an isotope ratio mass spectrometer (Delta plus Finnigan MAT, Bremen, Germany) coupled to an elemental analyser (NA2500 CE lnstruments, Rodano, Milano, ltaly) via an interface (Conflo lll Thermo Electron Cooperation. Bremen, Germany). Isotopic values are given as $\delta^{13}C$ values (standard: V-PDB): $\delta^{13}C$ (‰) = 1000 ($^{13}C/^{12}C_{sample}$ − $^{13}C/^{12}C_{standard})/(^{13}C/^{12}C_{standard})$.

Statistical analyses (ANOVA, repeated measures GLM per country, testing for normality and homogeneity of variances) were done with SPSS version 16.

Results and discussion

Average dry matter yields were significantly larger in Uganda than in Rwanda (Table 1). There were no significant differences in average biomass production among forage legumes or irrigation treatments; standard deviations were very large. The individual harvests showed large differences in biomass production within species (Fig. 1), with larger harvests in the rainy seasons (from March to May and September to November) than in the intermediate dry seasons, especially without extra irrigation. Individual harvests from Rwanda produced as much biomass as those from Uganda with similar variability (Fig. 1).

Table 1. Average dry matter yields of the five tested legumes over four (Rwanda) or five (Uganda) harvests (g m⁻²). There were significant differences between countries, but not among species or irrigation treatments within countries.

	L. purpureus	D. uncinatum	D. virgatus	M. bracteatum	C. brasiliensis
	Average dry matter yield [g m⁻²]				
Rwanda					
Irrigated	277	296	191	290	188
Not irrigated	280	285	316	309	385
Uganda					
Irrigated	701	530	602	542	704
Not irrigated	653	447	625	508	634

The carbon isotopic signatures were more enriched in samples from Rwanda than from Uganda (Fig. 1). This hints at a larger intrinsic water-use efficiency of the plants under conditions in Rwanda. As the biomass per harvest was not necessarily different between sites (Fig. 1), the larger efficiency was probably due to a smaller stomatal conductance, maybe due to climatic conditions or soil fertility. There were no clear relationships between biomass produced and $\delta^{13}C$ signatures for individual species, per country, per irrigation treatment or over all data. There were, however, significant differences in isotopic signatures among species within countries. In both countries, irrespective of irrigation, *C. brasiliensis* had most enriched isotopic values, while at the same time producing a relatively good harvest at most times (Figure 1). This suggests an efficient intrinsic water use of this forage legume that should be further investigated, especially regarding the variability of biomass production over time and its relation to the fertility level of the soils.
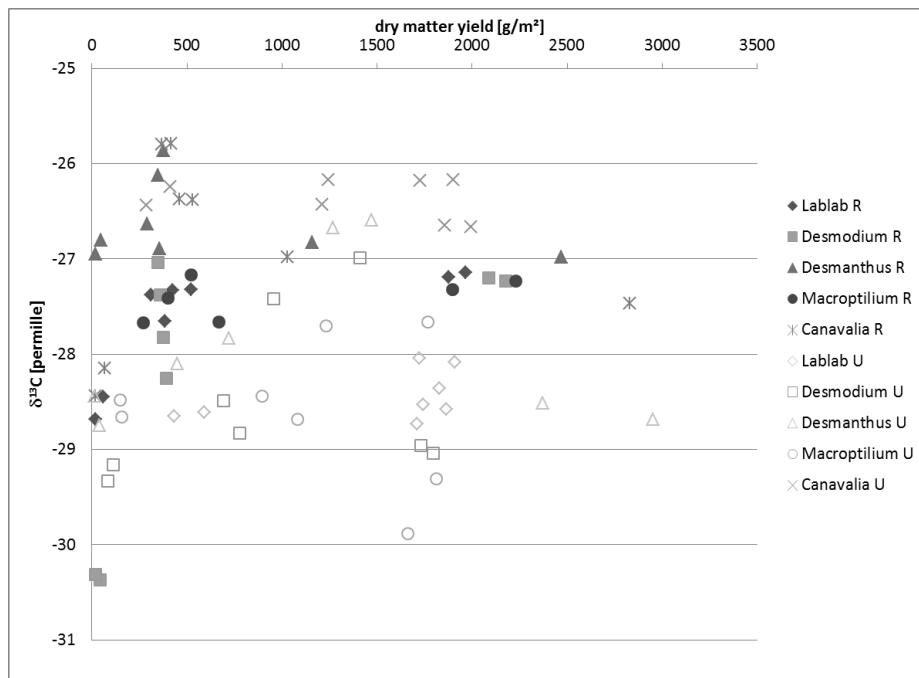
Figure 1. Carbon stable isotope signatures ($\delta^{13}C$ in ‰) versus dry matter yield (g m^{-2}) of five forage legumes over the first four harvests after sowing in Rwanda (R, closed symbols) and Uganda (U, open symbols). Shown are means per species and harvest with or without extra irrigation (eight points per species). *Macroptilium bracteatum* did not produce harvestable biomass during the first harvest in Rwanda

Conclusion

Despite a similar biomass production per individual harvest, stable carbon isotope signatures of the forage legumes tested were influenced by growing conditions in the countries, suggesting an influence on intrinsic water-use efficiency. Differences among species in biomass production and intrinsic water-use efficiency can be further exploited.

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Water use efficiency of tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) under different management intensity

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Abstract

Drought periods have been repeatedly observed in the last decade in the southern margin of the Alps. An adequate choice of drought-tolerant forage species and cultivars is of pivotal importance in tackling this challenge. Tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) are regarded as having contrasting levels of drought tolerance, but less is known about their intrinsic water use efficiency (W_i). To this end, analyses of the Carbon isotope composition of leaf material were performed on these species (one cultivar of perennial ryegrass, and two cultivars of tall fescue differing in leaf roughness) in the course of a field trial aiming at the optimization of seed mixtures for permanent meadows in drought-endangered areas under different management intensities and at two altitude ranges. W_i was found to be mainly affected by both genotype and altitude, with tall fescue showing higher W_i than perennial ryegrass.

Keywords: drought, *Festuca arundinacea*, *Lolium perenne*, intrinsic water-use efficiency

Introduction

Drought periods were repeatedly observed in the last decade in the southern margin of the Alps. The use of forage species and cultivars able to tolerate drought and efficiently use the water available from precipitation and irrigation represents an important issue for tackling this challenge. Perennial ryegrass (*Lolium perenne* L.) is considered to be well adapted to intensive management and to produce high-quality forage, but is also known to require adequate water availability, whereas tall fescue (*Festuca arundinacea* Schreb.) is regarded as a drought-tolerant grass with good yield potential, but with rapidly declining forage quality (Dietl *et al.,* 1998). Carbon isotope discrimination and water use efficiency are highly (negatively) correlated because both are affected by the relationship between photosynthesis and stomatal conductance. Hence, analysis of carbon isotope composition is a suitable tool to investigate intrinsic water use efficiency (W_i) in grassland. However, to our knowledge, no information at a species level is known for these grasses. For this reason, specific measurements were undertaken in the course of a field study in order to gain knowledge of W_i of tall fescue and perennial ryegrass. Specifically, the study tested whether the known difference in drought tolerance between the two species is related to a difference in W_i and whether differences in W_i are maintained in different environments, here at two altitudes and with different management intensities.

Materials and methods

Leaf material of tall fescue and perennial ryegrass was obtained from a field trial established three years before at two experimental sites (Table 1) and aiming at optimizing a seed mixture, containing both perennial ryegrass and tall fescue, for permanent mountain meadows at drought-endangered, non-irrigated locations. Three factors were studied in this experiment: seed mixture (Fa40 and Fa60, containing the same species, but 40% and 60% seed weight of tall fescue respectively), management intensity (low: 2 cuts year[-1] coupled to a fertilization

level equal to 2 livestock units ha^{-1}; high: 3 cuts year[-1] coupled to a fertilization level equal to 2.5 livestock units ha^{-1}) and the experimental site (low altitude: San Genesio/Jenesien 835 m a.s.l.; high altitude: Falzes/Pfalzen 1205 m a.s.l.). The experimental design is a Latin rectangle with 3 replications and a plot size of 4×4 m.

Table 1. Description of the experimental sites San Genesio/Jenesien and Falzes/Pfalzen.

Experimental site	Location	Geographic coordinates	Altitude (m a.s.l.)	Slope (%)	Aspect
Low altitude	San Genesio/Jenesien	46° 31' 25" N 11° 20' 22" E	835	26	S
High altitude	Falzes/Pfalzen	46° 49' 18" N 11° 53' 42" E	1205	31	S

Within each plot, three genotypes were sampled: a cultivar of perennial ryegrass (Ivana), a rough-leafed cultivar of tall fescue (Kora) and a soft-leafed cultivar of tall fescue (Barolex). Two different cultivars of tall fescue with contrasting leaf roughness (and equally abundant in the seed mixtures) were included in the study because differences in competitive ability, yield potential and some parameters of forage quality have been previously shown to be related to the leaf roughness of tall fescue (Peratoner *et al.*, 2010). On 28 and 29 August 2013 samples were taken at the high altitude and at the low altitude site, respectively. Each sample consisted of the youngest fully expanded leaf of 10 randomly selected plants per species and plot. The samples were oven-dried for 2 days at 60 °C and milled using a mortar mill (model RM 200, Retch, Haan, D). The samples were subsequently dried for 24 h at 40 °C, then amounts of 0.7 $\pm$ 0.05 mg were weighed into zinc cups and burned in an elemental analyzer (NA 1110, Carlo Erba, Milan, Italy) coupled to an isotope mass spectrometer (Delta Plus, Finnigan MAT, Bremen, D). As a control, a standard was measured with a known C/N ratio after every tenth sample. $^{13}C{:}^{12}C$ ratios of samples were used to compute W_i according to Köhler *et al.* (2010). Statistical data analysis was performed by means of a mixed model taking into account the genotype, the seed mixture, the management intensity and the design factors (lines and columns) as fixed factors. The genotype was considered to be a repeated factor with the plot as a subject. Post hoc comparisons were performed by Sidak test. A probability of $P<0.05$ was regarded as significant.

Results and discussion

W_i was found to be significantly affected by the genotype, the experimental site (both $P<0.001$) and also by their interaction ($P<0.01$). Tall fescue was found to have higher water use efficiency than perennial ryegrass at both sites, with the cultivar Barolex showing intermediate values between Kora and Ivana at the low altitude site (Table 2). This confirms field observations of better drought tolerance of tall fescue in comparison with perennial ryegrass, and contributes to explain these different attributes. $\square^{13}C$ was consistently lower at high altitude (i.e. carbon isotope discrimination was consistently higher at high altitude). This is in contrast with other findings which have shown a decrease of carbon isotope discrimination with altitude (e.g. Körner *et al.*, 1988; Männel *et al.*, 2007). However, these studies analysed different species at each altitude. Here, the same plant species were sampled, and thus, the observed change in W_i with altitude indicate the species response to a decrease in vapour pressure deficit. The altitudinal effect on W_i was stronger in perennial ryegrass than in both tall fescue cultivars. Again, this indicates a better adaption of tall fescue to conditions of high water demand.

Table 2. Intrinsic water use efficiency (µmol mol^{-1}) depending on genotype and management intensity at two contrasting altitudes. Means without upper case letters in common within each genotype and means without lower case letters in common within each experimental site significantly differ from each other.

Experimental site	Genotype (species, cultivar)			Management intensity	
	perennial ryegrass cv. Ivana	tall fescue cv. Barolex	tall fescue cv. Kora	low	high
Low altitude	85.6 [Ab]	88.6 [Aab]	92.4 [Aa]	90.3 [Aa]	87.4 [Aa]
High altitude	61.7 [Bb]	77.7 [Ba]	80.4 [Ba]	71.4 [Ba]	75.2 [Ba]

A further significant interaction was observed between experimental site and management intensity ($P<0.05$). Nutrient supply has indeed been demonstrated to affect W_i in grassland (Köhler *et al.*, 2012). However, while altitude effects on W_i were consistent with those already observed at the interaction genotype × experimental site, no significant difference depending on management intensity could be statistically detected by the post hoc test (Table 2). Another significant interaction was found between seed mixture and management intensity ($P<0.05$). The only significant difference was found at low management intensity, with the W_i of plots sown with Fa40 being higher than in the plots sown with Fa60 (83.4 µmol mol^{-1} and 78.4 µmol mol^{-1} respectively). Nutrient supply and differences in species composition have indeed been demonstrated to affect W_i in grassland (Köhler *et al.*, 2012). However, the explanation of these effects deserves further experimental work.

Conclusion

The present findings show that the higher drought tolerance of tall fescue relative to perennial ryegrass is related to a higher intrinsic water use efficiency. The species differences in W_i are consistent in environments differing in vapour pressure deficit.

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Important differences in yield responses to simulated drought among four species and across three sites

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Abstract

Summer droughts are predicted to increase in frequency due to climate change. We evaluated drought resistance in intensively managed grassland by using four model species (*Lolium perenne* L., *Cichorium intybus* L., *Trifolium repens* L., *Trifolium pratense* L.). The species represented different functional types, these being defined as a combination of traits related to symbiotic dinitrogen (N_2) fixation and rooting depth. A summer drought period of ten weeks with complete exclusion of precipitation was simulated in a common field experiment at three sites (Tänikon CH, Reckenholz CH, Wexford IE). Aboveground biomass production was impaired in the drought treatment at all three sites, the mean reduction (compared to a control) was 30% at Tänikon, 48% at Reckenholz, and 85% at Wexford. Different plant functional types varied in their drought resistance: N_2 fixing species showed only 8% and 28% biomass reduction at Tänikon and Reckenholz, respectively, compared to 51% and 68% for the non-fixing species. At Wexford, however, only the deep-rooted species *C. intybus* was able to counteract drought to some degree (57% biomass reduction compared to 94% reduction for the other three species). This suggests that the three sites exerted a very different degree of drought stress on plants but that cropping N_2 fixing and deep-rooted species can be an important management option under future climate conditions.

Keywords: drought, biomass production, plant functional types, intensive grassland

Introduction

In Central and Southern Europe, summer drought spells are expected to occur more frequently due to climate change (Lehner *et al*., 2006) and to impair forage production in grassland (Gilgen and Buchmann, 2009). Intensively managed grassland with high yielding forage species can be susceptible to drought and farmers might experience considerable loss of income (Finger *et al*., 2013). Therefore, the current forage production of intensively managed grassland should be adapted to future climate conditions, which could be achieved by cropping forage species with functional traits that improve drought resistance. Here, we present results from a multi-site drought stress experiment that aimed at studying four plant species from different functional types, these being defined as the factorial combination of traits associated with the method of nitrogen acquisition (N_2 fixing *vs.* non-fixing species) and the spatial pattern of root growth (deep- *vs.* shallow-rooted species). We hypothesized that N_2 fixing and deep-rooted species would have an improved drought resistance compared to non-fixing and shallow-rooted species. We also tested whether drought resistance of the four plant functional types would differ among sites.

Materials and methods

A common field experiment was set up at Tänikon and Reckenholz (northern Switzerland) and Wexford (south-east Ireland). At each site, monoculture plots (3 m × 5 m) of four plant species were established. The species represented the following functional types: a non-fixing,

shallow-rooted species (*Lolium perenne* L.), a non-fixing, deep-rooted species (*Cichorium intybus* L.), an N_2 fixing, shallow-rooted species (*Trifolium repens* L.) and an N_2 fixing, deep-rooted species (*Trifolium pratense* L.). A drought treatment was set up by simulating a summer drought period of ten weeks using rainout shelters (3 m × 5.5 m) that led to complete rain exclusion. The control treatment consisted of the actual climatic conditions and each combination of species and treatment was replicated three times per site. Aboveground biomass of the central strip (1.5 m × 5 m) of each plot was harvested six times per year (five at Wexford) at a cutting height of 7 cm (5 cm at Wexford) using a plot harvester. There were two re-growth periods during the drought treatment, and results from the second re-growth period are presented here. Differences in dry matter yield between control and drought treatment as well as among species were tested by analysis of variance (ANOVA).

Results and discussion

The simulated drought reduced aboveground biomass production significantly at all sites ($P <$ 0.001 each, Table 1).

Table 1: Mean (± s.e.) aboveground biomass production (kg DM ha^{-1} harvest^{-1}) of each of four forage plant species in second harvest period during simulated drought and the % reduction under drought *vs.* control conditions. Biomass production among species and between drought *vs.* control conditions were tested by ANOVA (ln-transformed). Three replicates per treatment.

	Tänikon CH			Reckenholz CH			Wexford IE		
	Monocultures			Monocultures			Monocultures		
	Control	Drought	%reduction	Control	Drought	%reduction	Control	Drought	%reduction
L. perenne	1355 (±98)	479 (±187)	65	682 (±78)	166 (±47)	75	668 (±146)	96 (±52)	86
C. intybus	1477 (±180)	935 (±78)	37	2062 (±143)	787 (±40)	61	808 (±30)	348 (±183)	57
T. repens	1763 (±34)	1523 (±74)	14	1197 (±129)	789 (±80)	34	1509 (±208)	33 (±19)	98
T. pratense	2841 (±103)	2791 (±150)	2	3232 (±193)	2551 (±259)	21	1013 (±115)	11 (±11)	99
Average			30			48			85
ANOVA									
Variable	Df	*F*-value	*P*-value		*F*-value	*P*-value		*F*-value	*P*-value
Species (Sp)	3	29.0	< 0.001		41.7	< 0.001		1.3	0.340
Drought (Dr)	1	18.7	< 0.001		31.1	< 0.001		68.1	< 0.001
Sp x Dr	3	6.3	0.005		8.9	0.006		4.3	0.043

The drought effect was moderate at both Swiss sites but severe at the Irish site. At Tänikon and Reckenholz, drought reduced the average biomass by 30% and 48%, respectively, whereas at Wexford the average reduction was 85%. The four different plant functional types varied in their drought resistance. At the two Swiss sites, aboveground biomass production of the N_2 fixing species *T. repens* and *T. pratense* was clearly less reduced under drought compared to that of the non-fixing species *L. perenne* and *C. intybus* (pooled contrasts: $P = 0.005$ at Tänikon, $P = 0.001$ at Reckenholz). Given the type of nitrogen acquisition (non-fixing or N_2 fixing), the deep-rooted species *C. intybus* and *T. pratense* also tended to be less impaired by drought than the shallow-rooted species *L. perenne* and *T. repens* ($P = 0.061$ at Reckenholz, $P = 0.104$ at Tänikon). However, at Wexford, only *C. intybus*, the species with the deepest roots, was able to counteract drought to some degree (57% biomass reduction) and it performed significantly better than the other three species ($P = 0.020$; contrast of *C. intybus vs.* the other three), which collapsed almost completely due to drought (94% biomass reduction on average).

It might be hypothesized that these differences in pattern of species' responses are related to the big differences in drought severity among sites. Under moderate drought, as at the Swiss sites, the N_2-fixing species have some growth advantage because they have access to N despite restricted uptake from dry soil due to symbiotic N_2 fixation. In contrast, the non-fixing species might suffer from reduced availability of mineral N under drought conditions. Such explanation would match results from previous studies investigating the drought response of plant functional types in temperate grassland (Lüscher *et al.*, 2005; Gilgen and Buchmann, 2009). Under severe drought stress, as at the Irish site, N_2 fixation is strongly impaired (Serraj *et al.*, 1999) and deep-rooted species might better resist the stress (Ho *et al.*, 2005, Gilgen *et al.*, 2010) if they can take up water from deeper soil layers. However, more detailed analyses are needed that will i) allow a more refined assessment of induced drought stress across different sites with varying soils and climatic conditions (Vicca *et al.*, 2012), ii) investigate the effect of drought severity on species' responses, and recovery rates, iii) reveal the effect of drought stress on symbiotic N_2 fixation, and iv) disentangle pure water limitation from a co-limitation of water and nutrients. Doing so should help identify traits for improved drought resistance and thereby selection of species for forage production under future climate conditions.

Conclusion

Our study showed varying species' responses related to differences in drought severity. At least under moderate drought, N_2 fixing as well as deep-rooted species showed mitigation of drought stress in intensively managed grassland. Cultivating mixtures including species from different plant functional types might therefore be a promising strategy to ensure future forage production under varying degree of drought severity.

Acknowledgements

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Assessment of the nutritive value and methanogenic potential of two cultivars of *Lotus corniculatus* L. and *Lotus uliginosus* Schkuhr.

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Abstract

The aim of this study was to compare the composition, *in vitro* gas production kinetics and methanogenic potential of *L. corniculatus* 'INIA Draco' and 'San Gabriel' and *L. uliginosus* 4n 'LE205' and 2n 'LE306'. Trefoils were seeded in a complete randomized block design with four replicates. Crude protein and neutral and acid detergent fibre were quantified. Gas production kinetics and methane production (from 0 to 8 and 8 to 24 h) were determined separately using an *in vitro* gas production procedure. Both cultivars of *L. uliginosus* presented higher ($P < 0.025$) fibre fractions, lower ($P < 0.010$) gas production kinetics and produced less methane in the overall period of incubation than cultivars of *L. corniculatus*. Within species, cultivars presented similar fibre contents and production of methane; however, differences ($P < 0.050$) in gas production kinetics were observed and *L. uliginosus* 'LE306' presented lower CP than did 'LE205'. Results of this study suggest that cultivars of *L. corniculatus* would provide higher degradable organic matter that would digest faster in the rumen than *L. uliginosus*. Differences between these two species in their methanogenic potential could be associated with differences in the amount and rate of fermentation of rumen degradable organic matter.

Keywords: birdsfoot trefoil, lotus major, chemical composition, *in vitro* gas production

Introduction

In Uruguay, sheep and beef nutrition is based mainly on grazing of native pastures. These pastures have a highly heterogeneous production, seasonal distribution, and quality of forage throughout the year. This implies periods of sub-nutrition, which negatively affects the performance of animals. Introduction of perennial legumes into native pastures, particularly *Lotus corniculatus* and *Lotus uliginosus*, has been widespread and has been used to improve the availability and forage-quality of production systems under grazing. These legume species have been of special interest because the condensed tannins in their foliage can provide significant benefits for ruminant performance, health and environmental sustainability (Waghorn, 2008). Most published information on these legumes has focussed primarily on biomass production and seasonal distribution, and there is no information in terms of feeding value and methanogenic potential of domestic cultivars. The aim of this study was to assess variation in chemical composition, *in vitro* gas production kinetics and methanogenic potential of *L. corniculatus* 'INIA Draco' and 'San Gabriel' and *L. uliginosus* 4n 'LE205' and 2n 'LE306'.

Materials and methods

Trefoils were seeded in 2008 at the National Institute of Agricultural Research 'INIA La Estanzuela', Colonia, Uruguay (34° 20' S 57° 41' W) in a complete randomized block design with 4 replicates. Forages were harvested on 27 October 2009. The phenologial stage was calculated using the criteria described by Hoffman *et al.* (1993). In all samples, total N (AOAC, 2007) and neutral and acid detergent fibre (with heat stable α-amylase in the neutral detergent

solution, expressed on an ash-free basis, aNDFom and ADFom, respectively) (Van Soest *et al.*, 1991) were quantified. Gas production kinetics and methane production were determined separately using an *in vitro* gas production procedure (Theodorou *et al.*, 1994). In this study the kinetics of gas production and the volume of gas produced were fitted using the NLIN PROC of SAS according to the model $V = a (1-e^{-kd\,(t-L)})$ where 'V' = cumulative gas production at time t, 'a' = potential gas production; 'kd' = fractional rate of gas production and "L" = lag time. No lag times were identified. The concentration of methane in the gas produced from 0 to 8 and 8 to 24 h of incubation was measured by gas chromatography. All results were analysed using the PROC MIXED of SAS considering total gas and methane accumulated from 0 to 8 and 8 to 24 hours of incubation as repeated measures.

Results and discussion

Cultivars of *L. corniculatus* and *L. uliginosus* 'LE205' were at a similar ($P > 0.80$) phenological stage, in the transition from late vegetative to late bud stage, while *L. uliginosus* 'LE306' was at a later (mid-bloom) ($P < 0.05$) phenological stage (Table 1).

Table 1. Phenological characterization, chemical composition (g kg DM^{-1}), *in vitro* potential of gas production (ml g iOM^{-1}) and fractional gas production rate (h^{-1}), and *in vitro* total gas (ml g iOM^{-1}) and methane (ml g iOM^{-1}) production of *Lotus corniculatus* ' INIA Draco' and 'San Gabriel' and *Lotus uliginosus* 'LE205' and 'LE206'.

Species	*Lotus corniculatus*		*Lotus uliginosus*		SEM	*P*
Cultivars	'INIA Draco'	'San Gabriel'	'LE205'	'LE306'		
PS	1.9[b]	2.1[b]	2.1[b]	3.2[a]	0.18	0.027
aNDFom, g kg DM^{-1}	446.7[b]	453.7[b]	508.7[a]	530.3[a]	15.5	0.009
ADFom, g kg DM^{-1}	321.7[b]	336.7[b]	380.0[a]	432.0[a]	16.5	0.012
CP, g kg DM^{-1}	183.3[a]	163.7[ab]	184.3[a]	140.0[b]	8.2	0.023
In vitro gas production kinetics						
'a', ml / g iOM	174.5[b]	186[a]	151[c]	159[c]	3.7	< 0.001
kd, h^{-1}	0.090[a]	0.087[a]	0.073[b]	0.066[b]	0.002	< 0.001
In vitro total gas and methane production						
Total gas (ml /g iOM) accumulated from:						
0 to 24 h	228[a]	224[a]	204[b]	189[c]	3.3	< 0.001
0 to 8 h	159[a]	156[a]	137[b]	121[c]	2.3	< 0.001
8 to 24 h	68	69	68	68	2.0	< 0.001
Methane accumulated (ml / g iOM) from:						
0 to 24 h	12.46[a]	13.29[a]	9.01[b]	7.54[b]	1.01	< 0.001
0 to 8 h	7.37[a]	6.88[a]	4.39[b]	3.84[b]	0.58	< 0.001
8 to 24 h	4.35[b]	6.35[a]	4.54[b]	3.51[b]	0.63	< 0.001

PS: phenological stage; aNDFom: amylase neutral detergent fibre (organic matter basis); ADFom: acid detergent fibre (organic matter basis); CP: crude protein; 'a': gas production potential; kd: fractional rate of gas production; iOM: incubated organic matter. a, b, c: In the rows, superscript letters indicate differences significant at $P \leq 0.05$.

Lotus corniculatus presented lower ($P < 0.025$) fibre fractions and higher ($P < 0.010$) gas production kinetics and methane production during the overall period of incubation (0 to 24 hours) than did *L. uliginosus*. The higher potential and rate of gas production in *L. corniculatus* suggested a greater degradability of the organic matter (OM) in the rumen. Both species produced high volumes of gas, and 64 to 70% of total gas was produced within the first 8 hours of incubation suggesting that their degradable OM would be fermented rapidly. Cultivars of *L. corniculatus* were similar ($P > 0.152$) in their chemical composition and methane production over the overall period of measurement; however, 'INIA Draco' had a lower ($P = 0.037$) potential of gas production than 'San Gabriel'. Cultivars of *L. uliginosus* presented similar ($P > 0.145$) fibre contents, gas production kinetics and overall methane production; however, 'LE306' presented lower ($P = 0.029$) CP than 'LE205'. The lower CP could be explained by

the difference in the phenological stage of cultivars. Buxton (1996) reported that protein concentration declines with increasing maturity because, in legumes, leaves presented higher protein content than stems and the proportion of leaves represents a smaller proportion of the available herbage in forages of more advanced maturity. During the overall period of measurement, cultivars of *L. corniculatus* produced 500 ml kg iOM^{-1} ($P < 0.035$) more methane than did cultivars of *L. uliginosus*. The production of methane is associated with the nature and rate of fermentation of carbohydrates, particularly with the carbohydrates of the cell wall, which are considered to be the most methanogenic. These carbohydrates are quantified as aNDFom, a fraction that has been positively associated with methane production (Moss *et al*., 2000). In this study, the aNDFom fraction in *L. uliginosus* was larger than in *L. corniculatus*; however, the former produced lower methane, suggesting that in these species the production of methane would be associated more with the amount and rate of degradation of the rumen degradable OM than to the aNDFom content. This could be the result of a higher content of more astringent condensed tannins in *L. uliginosus* which could inhibit fibre-degrading bacteria, reducing the proportion of dietary energy lost as methane (Waghorn, 2008).

Conclusions

Results of this study suggest that both the tested cultivars of *L. corniculatus* would provide higher degradable organic matter, which would degrade faster in the rumen than cultivars of *L. uliginosus*. Differences between species in their methanogenic potential could be associated with variations in the amount and rate of fermentation of rumen degradable organic matter.

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Improvement of the digestibility of tall fescue (*Festuca arundinacea* Schreb.) inspired by perennial ryegrass (*Lolium perenne* L.)

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Abstract

Due to climate change there is an increasing interest in NW Europe for more drought-tolerant fodder grass species like tall fescue (*Festuca arundinacea* Schreb.). Tall fescue has a high yield potential but a low digestibility. In a pot experiment we determined the digestibility and the sugar, fibre and protein content of 300 non-vernalized single plants (12 cultivars × 25 plants per cultivar) from perennial ryegrass and tall fescue. Tall fescue had a lower digestibility and sugar content and a higher fibre and protein content than perennial ryegrass. The variation in sugar content was as high for tall fescue as for perennial ryegrass. The same positive correlation between digestibility and sugar content was found in tall fescue as in perennial ryegrass. Because of the lower sugar content in tall fescue there seems to be enough margin to improve its digestibility by increasing the sugar content without adverse effects on fibre and protein value.

Keywords: *Festuca arundinacea*, *Lolium perenne*, digestibility

Introduction

Perennial ryegrass (*Lolium perenne* L.) is the most important fodder grass species on dairy farms in NW Europe. It is a high yielding species with a very good digestibility. However, during periods of drought, perennial ryegrass stops growing. Due to climate change more extreme weather conditions, including drought periods, are expected to occur (Parry *et al*., 2007). Therefore there is increasing interest in grass species that are more tolerant to drought stress. Tall fescue (*Festuca arundinacea* Schreb.) is not only more drought tolerant than perennial ryegrass but it also has a higher yield potential. Unfortunately, tall fescue lacks palatability and digestibility. For use on intensive dairy farms the feed quality of tall fescue needs to be improved. Leaves have a better digestibility than stems and therefore breeders should focus on the leaf component to improve digestibility (Beecher *et al*., 2013). Breeding leafy varieties without reheading is a first step. Also, in the vegetative stage there are differences in digestibility among varieties. In this study we compared the digestibility and its components of non-vernalized single plants of perennial ryegrass (Lp) and tall fescue (Fa) Based on this comparison we made some suggestions for the improvement of the digestibility of tall fescue.

Materials and methods

In February 2008, three hundred seeds of both Lp and Fa were sown in trays in the greenhouse. For each species, 25 seeds of each of 12 varieties or populations were used. In April 2008 the single plants were transplanted into 12 L pots, put on an open hardened field, and irrigated. An equalization cut was applied in May. Pots were further harvested in June and August. After each cut the pots were fertilized with 4 g of a 16-8-22 NPK fertilizer. At each harvest, fresh (FY) and dry weights of the single plants were measured and dry matter content (DMc) calculated. Dry matter digestibility (DMD) and contents of neutral detergent fibre (NDF), acid detergent fibre (ADF), water soluble carbohydrates (WSC) and crude protein (CP) were determined by NIRS (near infrared reflectance spectroscopy) on dried samples. The content of

protein digestible in the intestine (DP) was estimated by a multiple regression equation including DMD, CP, DM and NDF.

The perennial ryegrass plants showed no stems at all in the sowing year. About 20% of the tall fescue plants already showed one or a few real stems at the first harvest. At the second harvest more than half of the tall fescue plants had a few stems. Therefore we present results only from the first harvest.

Results and discussion

The vegetative tall fescue had, on average, significantly (t-test, $P<0.001$) lower digestibility and sugar content but a higher fibre and crude protein content than perennial ryegrass (Table 1). There was no difference in digestible protein content. The generative plants of tall fescue had an even lower digestibility and sugar content and higher fibre content. The variation of the feeding quality components among the single plants of both perennial ryegrass and tall fescue was highest for the sugar content and lowest for the digestibility.

Table 1. Means and coefficients of variation (cv) of fresh yield, dry matter content, digestibility, fibre, sugar and protein content for vegetative plants of perennial ryegrass (Lp) and both vegetative and generative plants of tall fescue (Fa).

	vegetative Lp (n=295)		vegetative Fa (n=230)		generative Fa (n=66)	
	mean	cv	mean	cv	mean	cv
FY (g)	116	18.2	87	23.5	121	23.1
DMc (%/FY)	26.1	7.7	24.0	8.1	24.6	6.6
DMD (%/DM)	79.4	3.9	76.4	3.6	71.0	5.5
NDF (%/DM)	37.8	7.9	41.3	6.9	46.5	7.7
ADF (%/DM)	18.9	9.8	18.7	9.2	22.6	11.7
WSC (%/DM)	33.3	13.0	27.9	16.2	25.4	16.9
CP (%/DM)	9.8	13.2	11.8	15.4	10.5	14.9
DP (%/DM)	8.4	4.4	8.4	5.9	7.8	7.7

The DMD was positively correlated with the WSC content and negatively with NDF and ADF (Table 2).

Table 2. Correlation coefficients between DMD and FY, DMc, NDF, ADF, WSC, CP and DP for vegetative plants of perennial ryegrass (Lp) and tall fescue (Fa)

	FY	DMc	NDF	ADF	WSC	CP	DP
Lp	0.09	0.51 ***	-0.92 ***	-0.81***	0.88***	-0.48***	0.67***
Fa	-0.17 **	0.41 ***	-0.84 ***	-0.79 ***	0.74 ***	-0.22 **	0.49 ***

In Figure 1 we ranked the plants from low to high digestibility and calculated trend lines for the other quality components. The course of the digestibility is parallel to the course of the WSC.

In perennial ryegrass the genetic variation for WSC is higher than for DMD (Humphreys, 1989). Thanks to this large genetic variation, high sugar ryegrasses have been bred and improvement in the energy value has been achieved by increasing the sugar content (Humphreys *et al.*, 2010). However, a very high WSC content in the grass may cause rumen acidosis. In tall fescue there is also a large variation in WSC. Because of the lower level of the WSC content in tall fescue there is a greater margin for improvement of the WSC without risk of acidosis. Also, the higher fibre content of tall fescue reduces this risk. WSC is often negatively correlated with CP (Ghesquiere *et al.*, 2007). As the CP content in tall fescue is higher than in perennial ryegrass the increase in WSC will have a less negative effect on the crude protein content and a positive effect on the protein use and on the reduction of the excreted nitrogen.
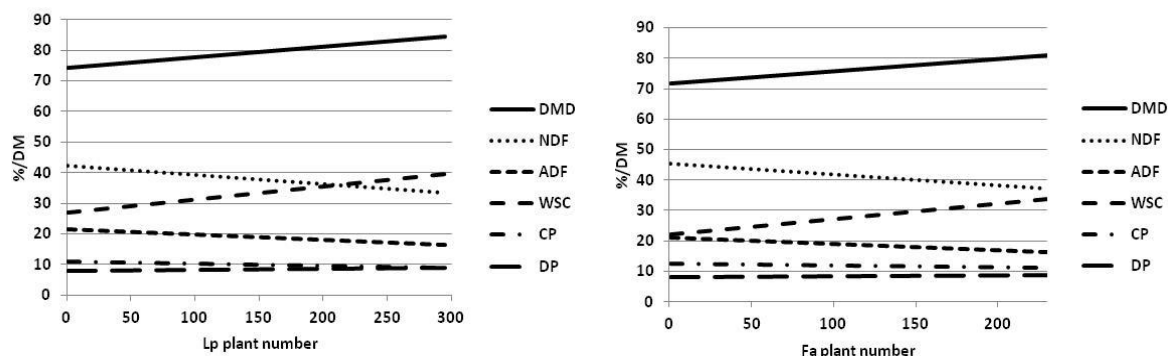
Figure 1. Trends of NDF, ADF, WSC, CP and DP for single plants of vegetative perennial ryegrass (left) and tall fescue (right) ranked from low to high digestibility.

Conclusion

The DMD in tall fescue is highly positively correlated with the WSC content, as is also the case in perennial ryegrass. There is also a lot of variation in WSC in tall fescue. As the average WSC content in tall fescue is much lower than in perennial ryegrass, there is scope for improving digestibility by increasing the WSC and also maintaining enough fibre and protein.

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Dry matter yield and digestibility of five cool season forage grass species under contrasting N fertilizations

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Abstract

The yield potential of different varieties of five grass species from the *Festuca* and *Lolium* genera were compared under two N-fertilization regimes (190 kg N ha^{-1} or 300 kg N ha^{-1} yr^{-1}) in two successive years under a cutting management. No N fertilization x species interaction was found. Hybrid ryegrass (*Lolium x boucheanum* Kunth.) was the highest-yielding species in the first year, and tall fescue (*Festuca arundinacea* Schreb.) in the second year. Meadow fescue (*Festuca pratensis* L.) was always the lowest-yielding species.

Keywords: ryegrass, fescue, *Festulolium*, N fertilization, digestibility

Introduction

Grassland farming in North-West Europe is changing. First, N-fertilization is decreasing, mainly due to legal restrictions (EU directive 91/676/EEC). Second, the importance of grazing in dairy farming is decreasing for different reasons (Van den Pol-van Dasselaar *et al.*, 2008), and consequently the importance of cut grass is increasing. Third, more dry summer spells are expected due to climate change. As there are differences in N-use efficiency (Gilliland *et al.*, 2010) and in drought resistance of different forage grass species (Willman *et al.*, 1998) it is pertinent to revaluate the performance of perennial ryegrass (*Lolium perenne* L.; Lp) - currently the most widely used species - in comparison with more drought-tolerant species that perform well under a cutting management. The aim of this trial was to compare the performance of five forage-grass species under two N levels in a cutting management. The hypothesis was that there is an N-fertilization × species interaction for the different species.

Materials and methods

A trial was established in September 2011 on a sandy loam soil in Merelbeke, Belgium, comprising two varieties of Lp ('Indiana' and 'Toronto'), tall fescue (*Festuca arundinacea* Schreb.; Fa; 'Barolex' and 'Callina'), hybrid ryegrass (*Lolium* x *boucheanum* Kunth.; Lh; 'Melprius' and a candivar), meadow fescue (*Festuca pratensis* L., Fp;, 'Pardus' and a candivar) and *Festulolium* (Fl; 'Achilles' and 'Lueur'). The trial was a split-plot design with three replicates; individual plot size was 7.8 m². N fertilization was the main plot factor with two levels: high (300 kg N ha^{-1} yr^{-1}) or low N (190 kg N ha^{-1} yr^{-1}) and the varieties of the different species formed the subplot factor. Plots were sown at a density of 10000 germinable seeds per plot. P and K fertilization for high and low N fertilizations were respectively: 16 and 11 kg P ha^{-1} and 267 and 189 kg K ha^{-1}. Five cuts were harvested, in both 2012 and 2013. The fresh yield was determined by harvesting the entire plot using a plot harvester (Haldrup, Logstor, Denmark). A random subsample of the harvested material was taken from each plot and dried for at least 24 h at 70 °C. Digestibility of all varieties was analysed with NIRS (calibrated with Tilley and Terry) on a single pooled sample per variety and cut in 2012.

ANOVA of the dry matter yields (DMY) for both years were performed using the *aov()* function in R. The hierarchy of the split-plot design and the nesting of the varieties within species were taken into account in the model. Multiple comparisons of species averages within the N levels was performed using the *TukeyHSD()* function.

Results and discussion

There was, obviously, a positive effect of N-fertilization on the DMY ($P < 0.01$) in both years and there was also a significant species effect on DMY in both years ($P < 0.01$). There was no significant species $\times$ N fertilization interaction, either in 2012 ($P = 0.43$) or in 2013 ($P = 0.17$) and therefore the DMY ranking of the species did not change when N fertilization was increased from 190 kg N ha^{-1} yr^{-1} to 300 kg N ha^{-1} yr^{-1} (Figure 1).
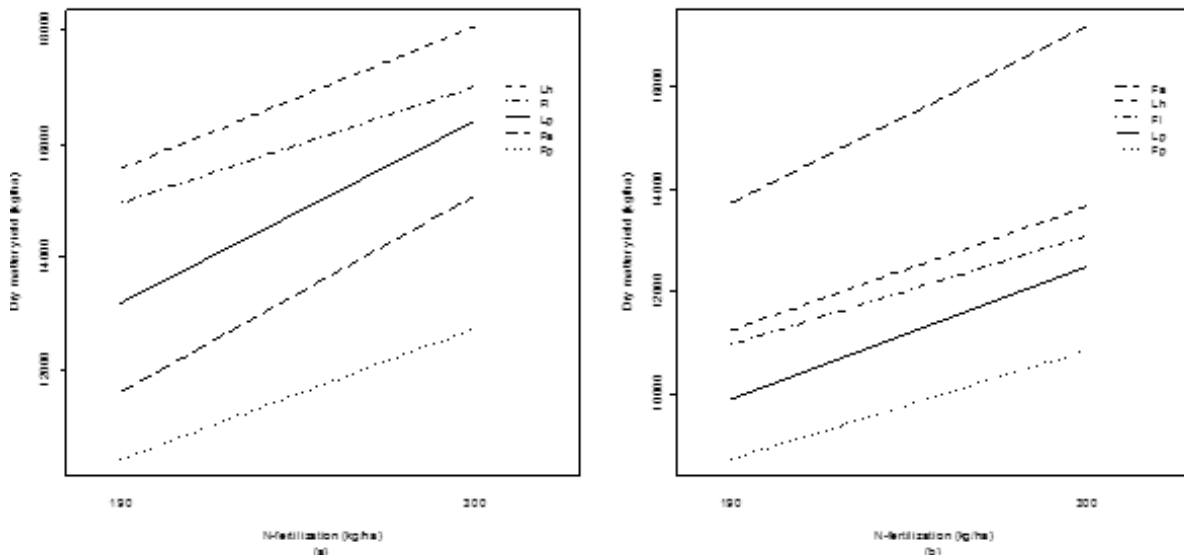
Figure 1. Interaction plots for dry matter yields (kg ha^{-1} yr^{-1}) of *Lolium* x *boucheanum* (Lh), *Festulolium* (Fl), *Lolium perenne* (Lp), *Festuca arundinacea* (Fa) and *Festuca pratensis* (Fp) averaged over two varieties per species at two N fertilization levels in 2012 (a) and 2013 (b).

In 2012, the first year after establishment, Lh and Fl were overyielding Fp and Fa at both N fertilizations (Figure 2a).
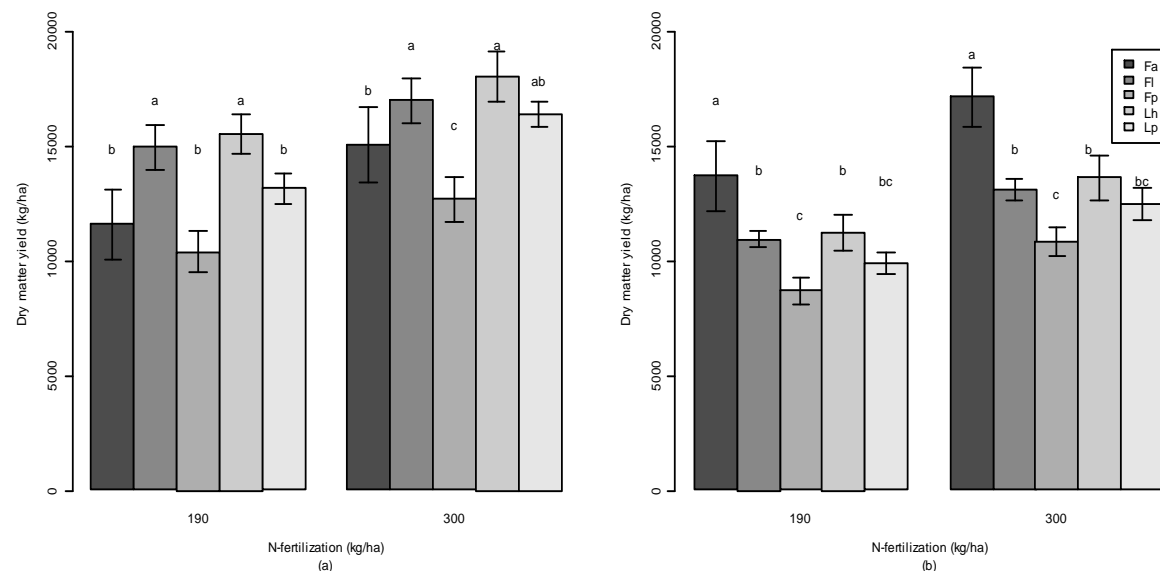
Figure 2. Dry matter yield (kg ha^{-1} yr^{-1}) of *Lolium* x *boucheanum* (Lh), *Festulolium* (Fl), *Lolium perenne* (Lp), *Festuca arundinacea* (Fa) and *Festuca pratensis* (Fp) averaged over two varieties per species at two N fertilization levels in 2012 (a) and in 2013 (b). Error bars: $\pm$ standard deviation. Bars with a different letter are significantly different ($P < 0.05$; Tukey test).

Averaged over both N levels, the DMY of Lh, the highest yielding species, was 1.49 and 1.34 times the DMY of Fp and Fa respectively. In 2013, the second full production year, Fa overyielded all the other species at both N fertilizations (Figure 2b). Fp remained the lowest

yielding species. Although the yield of Lh and Fl remained superior to that of Lp in the second year, their sward densities started to decline.

The yield pattern of Fa was in accordance with earlier observations: due to its slow early vigour it has a low yield in the first production year, but once established it is very high yielding and persistent (Cougnon *et al.*, 2013). The yield deficit of Fa in the first year was compensated by its high yield in the second year: averaged over the two years and over the two N levels, DM yields of Fa, Lh and F1 were not significantly different (28796, 29277 and 28030 kg DM ha^{-1} respectively). The tested Lh and Fl varieties seemed appropriate for leys lasting for only 2-3 years. Fa had the right properties to become an important forage grass in long-lasting cut grassland: once established it had the highest yield potential. The absence of drought periods in the experimental period did not allow any confirmation of the supposed superior drought resistance of *Festuca* genes. The digestibility of Fa was consistently lower than that of Lp and Lh, while the digestibility of Lh was generally higher than that of Fl (Table 1).

Table 1. Organic matter digestibility (%) in the first full production year. Method: NIRS (cal. Tilley and Terry); data are weighed averages of 5 cuts. (Species codes: see Figure 1.)

	Fa	Fl	Lh	Fp	Lp
High N	72.1	75.1	79.3	80.1	79.8
Low N	73.0	76.5	81.0	79.4	80.7

Conclusions

Our hypothesis was not confirmed: there was no N fertilization × species interaction for the tested species. Meadow fescue always had the lowest yield performance, *Festulolium* and hybrid ryegrass varieties were the winners in year 1, and tall fescue was the winner in year 2.

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Grazing season length on dairy, beef and sheep farms in Europe

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Abstract

Grazing season length and livestock stocking density on grassland are two important parameters for modelling grassland processes such as nutrient losses and parasite transmission. However, there is a lack of data available on these two important parameters for most European grazing systems. This study calculated weighted means of grazing season lengths and grazing stocking rates for dairy, beef and sheep farms in 986 regions in 32 European countries using results from the 2010 Eurostat Survey on Agriculture Production Methods (SAPM). Means for each country are presented here.

Keywords: grazing, pasture, livestock density

Introduction

Grazing season length has particular implications for production costs, nutrient losses, greenhouse gas emissions and parasite transmission. For example, extending the grazing season may reduce production costs, increase nutrient losses and increase *Ostertagia* infection levels (Charlier *et al.,* 2005; Webb *et al*., 2005; Dillon *et al.,* 2008). However, data on grazing season length in various regions and farm systems are generally not available. The EU GLOWORM project (www.gloworm.eu) is modelling the effects of future climate change on grazing management and parasite transmission. This requires data on current grazing season lengths on farms across Europe. The objective of this study was to establish a database of grazing-season length and grazing-livestock stocking rates on grassland across Europe.

Materials and methods

Data on grazing season length were obtained from the 2010 Survey on Agricultural Production Methods (SAPM). The survey was conducted on a sample population (between 3% and 30%) of farms in Belgium, Cyprus, Croatia, Denmark, Finland, Germany, Greece, Hungary, Ireland, Latvia, Norway, Poland, Slovenia, Spain, Sweden, Switzerland and United Kingdom. It was conducted as part of farm census collection in Austria, Bulgaria, Czech Republic, Estonia, France, Iceland, Italy, Lithuania, Luxembourg, Malta, Montenegro, Netherlands, Portugal, Romania and Slovakia. Full SAPM methodological details are available at: http://epp.eurostat.ec.europa.eu/statistics_explained/index.php. Farm enterprises were classified as dairy, beef or sheep farms according to standard EU farm typology (EU Commission Regulation No 1242/2008). The collated SAPM data was obtained in categorical format with the six following categories for 'number of months with livestock grazing': 0 months, 1 to 2 months, 3 to 4 months, 5 to 6 months, 7 to 9 months and 10 months or more. This study calculated weighted means and weighted standard deviations (stdev) from the proportion of LU in each category for both on-farm grazing and commonage grazing for each farm system and region using the following equations:

(i) weighted mean = category mean × (proportion of farms in each category ÷ total farms)

(ii) weighted stdev = (category mean - weighted mean)^2 × (proportion of farms in each category ÷ total farms)

Category means and farm type were not available for area of commonage. Therefore a total stocking rate was used (total grazing LU of farms that practised common grazing / total area of commonage (ha)) in place of category means in the above equations for common land.

Results and discussion

Calculated grazing season lengths and grazing stocking densities for each country are shown in Table 1. Creighton *et al.* (2011) conducted a questionnaire survey of dairy farmers in Ireland and found that farmers grazed their cows for approximately 8.6 months, similar to the 8.8 months for Irish Dairy farms in Table 1.

Table 1. Grazing season length and stocking density on grazed grassland for dairy, beef and sheep farms across Europe. Standard deviations are shown in parenthesis.

		Grazing season length (months)			Grazing stocking density (LU ha^{-1})		
		Dairy	Beef	Sheep	Dairy	Beef	Sheep
AT	Austria	4.1 (2.49)	4.0 (3.01)	5.6 (3.03)	1.6 (0.67)	1 (0.56)	0.9 (0.52)
BE	Belgium	6.8 (1.79)	6.3 (2.48)	6.4 (3.33)	6.2 (10.36)	4.8 (8.02)	3.4 (5.26)
BG	Bulgaria	8.0 (2.00)	8.7 (2.38)	8.4 (2.14)	0.7 (0.81)	0.4 (0.60)	0.3 (0.39)
CH	Switzerland	5.8 (2.30)	5.5 (2.18)	4.8 (1.77)	2.3 (2.00)*	2.1 (1.47)*	1.1 (1.18)*
CY	Cyprus			2.2 (3.79)			6.0 (6.82)
CZ	Czech Republic	4.3 (3.19)	7.7 (2.79)	8.6 (2.54)	3.1 (1.21)	0.7 (0.07)	0.8 (0.03)
DE	Germany	4.1 (3.25)	4.9 (4.13)	5.3 (4.71)	4.0 (0.74)	1.5 (0.28)	0.9 (0.10)
DK	Denmark	4.3 (2.94)	6.0 (3.76)	7.3 (3.94)	7.8 (2.48)	3.3 (1.02)	1.6 (0.30)
EE	Estonia	4.7 (1.51)	6.7 (1.61)	6.7 (1.55)	2.7 (0.79)	0.8 (0.07)	0.6 (0.07)
EL	Greece	4.9 (4.16)	9.1 (2.63)	8.9 (2.79)	0.7 (1.51)	1.0 (3.03)	0.6 (0.48)
ES	Spain	5.5 (4.77)	9.1 (2.93)	9.4 (2.77)	3.0 (1.48)	0.9 (0.48)	0.7 (0.33)
FI	Finland	3.3 (2.03)	2.9 (3.26)	4.3 (2.91)	5.4 (1.98)	3.4 (1.83)	1.7 (0.23)
FR	France	8.8 (1.82)	9.3 (1.73)	9.4 (2.22)	2.1 (0.35)	1.2 (0.18)	0.7 (0.17)
HR	Croatia	3.6 (3.78)	2.0 (3.45)	9.0 (2.37)	1.7 (1.12)	1.0 (0.86)	0.2 (0.72)
HU	Hungary	2.7 (3.21)	5.1 (3.90)	5.6 (3.71)	4.6 (6.48)	0.7 (0.59)	0.8 (0.32)
IE	Ireland	8.8 (1.60)	8.1 (1.72)	9.5 (2.30)	2.2 (0.26)	1.2 (0.19)	0.7 (0.28)
IS	Iceland	6.3 (2.55)	7.2 (2.90)	6.4 (2.78)	0.6 (0.15)	0.3 (0.18)	0.1 (0.03)
IT	Italy	1.2 (2.66)	3.6 (4.55)	8.4 (3.76)	1.8 (1.12)	0.9 (0.58)	0.9 (0.37)
LT	Lithuania	5.2 (1.48)	5.0 (1.98)	5.3 (1.95)	1.1 (0.22)	1.0 (0.04)	0.7 (0.05)
LU	Luxembourg	6.8 (1.25)	6.9 (1.68)	6.6 (1.61)	3.1 (0.06)	2.3 (0.10)	1.6 (0.24)
LV	Latvia	5.6 (2.07)	7.1 (2.48)	6.4 (2.28)	1.0 (0.07)	0.6 (0.04)	0.7 (0.07)
ME	Montenegro	3.3 (3.14)	1.8 (2.76)	3.7 (3.27)	1.2 (0.24)	0.9 (0.15)	1.9 (0.73)
MT	Malta			0.9 (2.62)			*
NL	Netherlands	5.3 (2.71)	4.0 (3.95)	6.2 (4.21)	3.9 (1.03)	3.8 (1.39)	2.4 (0.61)
NO	Norway	2.6 (1.79)	3.4 (2.14)	3.5 (2.26)	3.6 (0.57)	2.6 (0.25)	2.0 (0.23)
PL	Poland	3.9 (2.63)	3.6 (3.11)	5.3 (2.36)	3.9 (1.07)	1.8 (0.69)	1.3 (0.37)
PT	Portugal	5.2 (5.36)	9.7 (3.22)	9.9 (2.75)	3.1 (2.94)	0.6 (0.33)	0.5 (0.32)
RO	Romania	4.6 (2.63)	4.2 (3.16)	5.8 (2.53)	0.7 (0.79)	0.5 (0.68)	0.8 (0.71)
SE	Sweden	5.6 (1.59)	6.3 (2.04)	6.7 (3.02)	4.4 (1.20)	1.9 (0.20)	1.0 (0.13)
SI	Slovenia	3.2 (3.46)	3.5 (3.36)	6.2 (2.79)	4.7 (1.50)	2.1 (0.56)	1.3 (0.42)
SK	Slovakia	4.7 (2.58)	4.3 (2.43)	6.0 (2.11)	1.3 (0.34)	0.6 (0.05)	0.5 (0.05)
UK	United	8.1 (2.23)	8.6 (2.51)	10.0 (2.39)	2.8 (0.22)	1.4 (0.23)	0.8 (0.13)

* Commonage grazing land area not available, on-farm stocking rate only.

Kuusela (2004) stated that the grazing season in Finland was approximately 3-5 months, and the results in Table 1 for Finland are within that range. In Austria, Steinwidder *et al.* (2010) recorded grazing season lengths on six pilot dairy farms ranging from 5 to 7 months, this is considerably longer than the 4.1 months recorded here (Table 1), possibly because the focus of the former study was on developing low-input farming methods. In northern Spain, Mendarte *et al.* (2010) stated that grazing season length in the area started in May and ended in October, giving a grazing season length of approximately six months, similar to the results for dairy farms in this study (Table 1).

One of the limitations of this study is that the data are derived from a categorical, rather than discrete dataset (see Materials and methods section). Therefore, within-category variation and distribution is not available. While this is not a major issue at country level, due to the large number of results and wide spread across categories, at the much smaller NUTS 3 level it can have a larger effect. Another limitation is that daily grazing duration is not accounted for. For example, a questionnaire survey in 2006 found that the percentage of farms that grazed dairy cows both day and night during the summer in Belgium, Germany, Sweden, Ireland and the UK, were 58, 26, 71, 98 and 87%, respectively (Bennema *et al.,* 2010). Finally, the results are only for one year (2009) and therefore any anomalies (e.g. weather) within that year for each region need to be considered when interpreting the data.

Conclusion

Weighted mean grazing season length and grazing stocking density were calculated for dairy, beef and sheep farms in 986 NUTS 3 regions in 32 countries across Europe. Results for each country are presented in Table 1. Grazing season length varied widely between regions and farm types. This data set is currently being used to assess the potential future impacts of climate change on grazing management across Europe.

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Effects of mild heat stress periods on milk production, milking frequency and rumination time of grazing dairy cows milked by a mobile automatic system

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Abstract

Grazing dairy cows milked by an automatic system (AS) experienced mild heat stress (HS) periods twice during the summer. The daily temperature humidity index (THI) during these periods were higher than 72. Milk production, as well as milking frequency, rumination time and milk fat to protein ratio (F/P) during these periods were compared with adjacent periods with mean THI of 61. The daily milking frequency, the total number of visits to the AS, and milk production were significantly higher in HS periods (2.12 vs. 1.97, 2.99 vs. 2.69, and 19.7 vs. 18.5 kg milk per cow, respectively). There were significant interactions between times and periods for milking frequency and number of visits, whereas the daily rumination time was significantly lower (339 vs. 419 min) and the F/P in milk tended to be decreased (1.17 vs. 1.23). These results could be explained by changes in cow behaviour during HS periods.

Keywords: dairy cows, grazing, automatic milking system, heat stress, rumination

Introduction

Cows milked by automatic systems (AS) are most often confined indoors or have access to pasture only during the day in summer. However, grazing allows reduced feeding costs and it improves animal health and welfare. A mobile AS, as described by Dufrasne *et al.* (2010), allowing grazing of dairy cows in fragmented areas is thus advantageous for animals. At grazing, the cows can move more than when in the barn and they are exposed to the environmental conditions. During heat stress periods, it is known that feed intake can be reduced, especially when temperatures are above 25 or 26 °C (Rhoads *et al.*, 2013). Little information exists on the effects of heat stress on grazing dairy cows milked by an AS. The aim of this study was to determine the effects of heat stress periods on the milk yield, milking frequency, fat to protein ratio in milk (F/P) and rumination time (RT) of grazing dairy cows milked by an AS located on the pasture.

Materials and methods

This study was carried out at the Experimental Farm of the University of Liège (Belgium). A herd of about 50 dairy cows grazed on 18 ha of permanent pastures and was milked by an AS Lely A3 next®. The grazing period began in April and ended in October. The cows grazed by strip grazing and two allocations per day were provided. The gate of the AS was manually changed twice per day, at 6:00 h and 16:00 h, to guide the cows on to the next allocation. The cows had to pass in the AS in order to benefit from the new allocation. In practice, they were fetched for the morning milking, allowing a daily survey of the animals by the herdsman. They came freely to access the AS when the gate was changed at the afternoon. Furthermore, they had free access to the AS at day and night times. The temperature humidity indexes (THI) were calculated according to Ingraham *et al.* (1979) and were used to define, *post hoc,* mild heat stress periods (HS) according to Armstrong (1994). Consequently, a period of 4 and 7 consecutive days were identified in July (J) and in August (A), respectively. These periods were characterized by a THI >72 during the day and 23.1 °C mean temperature. These two heat stress periods were compared to corresponding normal periods chosen close before and after

the period of heat stress - less than 9 days - for their similarity regarding the distance from the AMS and the availability in water in the grazed paddock. During normal periods, maximum THI was < 68 and mean temperature 16.3 °C. Water was always available near the AS in a tin (1000 L) and available in some grazed paddocks in individual bowls. Only animals present from the beginning till the end of these periods were taken into account. The total number of lactating cows was 45 in J and 47 in A. The lactation number and days in milk during the normal and HS periods were similar (Table 1). The pastures consisted mainly of perennial ryegrass and white clover. The grass heights were measured by an INRA rising plate meter at each entry and exit in the paddocks. Grass yield was measured with a mower, cutting strips of 10 meters long. Grass was sampled at each entry in order to determine chemical composition. Each cow received an amount of concentrate determined with respect to lactation stage. The cows were equipped with a HR-Tag neck collar recording rumination parameters and cow activity (SCR, Israel). The temperature, THI, distance from the paddock to the AMS, days in milk and lactation number were analysed according to a GLM by using THI conditions (normal and HS) and periods (J and A). Data electronically captured by the AS, i.e. milk production, milking frequency, milking visits (result of the sum of milkings, failed and refused milkings), F/P and RT (991 data) were analysed according a mixed model (SAS, 1999) including THI conditions, periods, lactation number, stages of lactation as fixed effects, and allowing an type 1 autoregressive covariance structure for measurements performed on each animal within THI conditions and periods.

Results and discussion

THI and temperature were significantly different and there was no interaction between THI conditions and periods for environmental parameters (Table 1).

Table1. Some environmental and animal characteristics during periods of normal and heat stress conditions in lactating cows at pasture (mean and standard deviation)

	Temperature (°C)	THI	Distance (m)	Days in milk	Lactation number
Normal	16.3±0.9	60.8± 1.4	190.5 ±79.1	183.5± 89.4	2.83± 1.78
Heat stress	23.1± 2.3	70.5± 2.9	187.8 ±40.6	182.9± 89.5	2.84 ±1.77
P value	<0.0001	<0.0001	NS	NS	NS

The grass composition in terms of crude protein, neutral detergent fibre, acid detergent fibre, water soluble carbohydrate (% in DM) and grass digestibility (%) were 16.3, 47.8, 27.3 and 79.7 in J, and 19.2, 49.6, 26.2 and 80.6 in A. The grass heights were 10.1 and 8.5 cm at entry and 3.1 and 3.1 cm at the exit, respectively in J and A respectively. The grass yield was 1509 and 1437 kg DM ha^{-1} in J and A respectively, the calculated sward availability was 14 kg DM per day and per cow. On average, the cows received 1.9 kg and 2.0 kg concentrate per day during normal and HS periods. Milking frequency and visits were significantly higher in HS (Table 2). There were significant interactions between THI conditions and periods, the longest periods in A showing no difference between N and HS (1.90 vs 1.98 milking per day and 2.67 vs. 2.51 milking visits respectively). The higher milk production in HS periods can be explained by the increased milking frequency. The cows were attracted to the AS to drink water from a large trough located near the AS during HS periods (unpublished observations). These observations did not confirm those of Spörndly and Wredle (2005) who reported no significant difference in milk yield, milking frequency or water intake between a group of cows with unlimited access to water and a group that had access to water only in the barn. In the present study, with an increase in THI, this behaviour probably increased the number of milking visits and milking frequency. The daily RT was considerably decreased in HS. A reduction of the RT

in cows suffering from mild to moderate heat stress in the barn was reported by Soriani *et al.* (2013).

Table 2. Daily parameters recorded in cows exposed to normal and mild heat stress at pasture (mean and standard error).

	Milking frequency	Milking visit	Milk prod. (kg day-1)	Rumination (min day-1)	Milk F/P
Normal	1.97±0.04	2.69±0.09	18.5±0.3	418.9± 8.7	1.23± 0.01
Heat stress	2.12± 0.04	2.99±0.09	19.7±0.4	339.2± 9.5	1.17 ±0.01
P value	<0.01	<0.05	<0.01	<0.0001	<0.10

Decrease in RT is often associated to a reduction in dry matter intake. It seems that, within the conditions of this trial, this was not observed as the milk yield in HS cows was not reduced, and was even increased. F/P tended to be lower in HS. This can be related to a diminution in pH rumen explained by a decrease in saliva production resulting from RT reduction. Such a decrease in ruminal pH when environmental temperature was increased was also described by Mishra *et al*. (1970).

Conclusion

It appears from these results that rumination, milking frequency and milk performance of cows milked by an automatic system are affected by a mild heat stress at pasture. More studies are needed to study the impact of the length of HS on these parameters.

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Interactive effects of *Epichloë* endophytes and plant origin on mineral content in *Festuca rubra*

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Abstract

Red fescue (*Festuca rubra*) is a perennial grass growing in a wide range of ecological conditions in grasslands. It is often asymptomatically infected by the systemic fungal endophyte *Epichloë festucae* in European grasslands. In this study we analysed the mineral concentration of infected and uninfected *F. rubra* plants collected from southern (Spain) and northern Europe (Faroe Islands and Finland), and grown in an experimental field in Spain. Our results showed differences in mineral concentrations between plants collected from different geographic locations and endophyte status of the plants. The endophyte increased the concentration of several nutrients in the plants from northern Finland. The northern fescue plants from Faroe and Finland had, on average, a greater concentration of most mineral elements (P, K, Ca, Mg, S, Fe, Mn, Cu, Ni, Na, Al and Cr) compared to plants from Spain.

Keywords: red fescue, macronutrient, micronutrient, fungal endophytes

Introduction

Festuca rubra L. is a perennial grass very persistent and tolerant to a wide range of ecological conditions. This grass species is asymptomatically infected by the systemic fungal endophyte *Epichlöe festucae* across European grasslands from Spain (Zabalgogeazgoa *et al*., 1999) to northernmost Finland and Norway (Wäli *et al.,* 2007). *Neotyphodium* and *Epichloë* endophytes are seed transmitted and can be beneficial for host grasses. Endophytes may increase herbivore resistance, and the tolerance of the host grass to abiotic stresses (Malinowski and Belesky, 2006). In some circumstances epichloid endophytes can increase the nutrient content of infected plants (Malinowski *et al*., 2000; Zabalgogeazcoa *et al*., 2006; Vázquez de Aldana *et al*., 2013). The effects of the endophyte on the host plant are variable and dependent on the fungal and plant genotypes as well as environmental conditions (Saikkonen *et al.*, 1998).
The objective of the present study was to determine the effect of *Epichloë* endophyte on the mineral content of *F. rubra* plants originally collected from different geographic locations across Europe, when grown under the same environmental conditions.

Materials and methods

Festuca rubra plants were collected from grasslands in North and South Europe: Faroe Islands, northern Finland (Utsjoki), and western Spain (Salamanca) in 2011. At each location plants were collected from two *F. rubra* populations and transported to the laboratory. The presence of the fungus *Epichloë festucae* in plants was verified by isolating the fungus from stem and leaf sheaths on potato and dextrose agar (Bacon and White, 1994). The frequencies of endophyte-infected plants were in the ranges of 40-80%, 4-71% and 52-68%, in Spain, Faroe Islands and northern Finland, respectively.

Endophyte-infected (E+; n=95) and uninfected (E−; n=58) plants were transplanted in an experimental field in Spain (Salamanca) in 2012. The soil type in the field is an eutric chromic cambisol soil with neutral pH at the surface. Plants were watered during the first weeks from October to November to promote their establishment. In June 2013, aboveground biomass of

plants was harvested. Dried and ground samples were calcined (450 °C) and ashes dissolved in HCl:HNO$_3$:H$_2$O (1:1:8). The concentrations of mineral elements (P, K, Ca Mg, S, Fe, Mn, Zn, Cu, Na, Al, Co and Cr) were determined by inductively coupled plasma (ICP) spectroscopy. Differences between infected and uninfected plants were tested by one-way ANOVA for each of the three locations.

Results and discussion

Mineral concentrations between geographic plant origins and endophyte status of the plants differed. The endophyte effect on the mineral concentration was significant for several elements and origins but not for biomass production (Table 1).

Table 1. Mineral content in endophyte infected (E+) and non-infected (E-) plants of *Festuca rubra* from North (Faroe Islands and Finland) and South Europe (Spain).

Element	Faroe Islands			Finland			Spain		
	E-	E+	*P*	E-	E+	*P*	E-	E+	*P*
Essentials									
P (g kg^{-1})	3.49	4.42	0.341	**3.26**	**3.73**	**0.060**	2.74	2.84	0.584
K (g kg^{-1})	9.13	12.9	0.387	**9.79**	**12.1**	**0.007**	7.27	7.08	0.487
Ca (g kg^{-1})	2.51	2.91	0.978	**2.50**	**3.10**	**0.009**	1.67	1.63	0.991
Mg (g kg^{-1})	0.855	0.965	0.787	0.801	0.887	0.141	0.635	0.653	0.533
S (g kg^{-1})	1.20	1.39	0.348	**0.975**	**1.14**	**0.040**	855	854	0.817
Fe (mg kg^{-1})	**349**	**204**	**0.032**	**231**	**281**	**0.050**	132	136	0.313
Mn (mg kg^{-1})	42.4	42.2	0.322	39.5	43.3	0.262	34.9	31.3	0.457
Zn (mg kg^{-1})	21.8	20.4	0.552	**19.1**	**21.5**	**0.078**	18.5	19.6	0.318
Cu (mg kg^{-1})	6.29	8.08	0.737	**5.35**	**6.70**	**0.005**	4.37	4.29	0.879
Ni (mg kg^{-1})	3.55	3.06	0.518	2.60	2.54	0.828	1.80	2.02	0.103
Beneficials									
Na (g kg^{-1})	0.291	0.253	0.298	0.185	0.196	0.352	0.208	0.209	0.783
Al (mg kg^{-1})	**426**	**180**	**0.011**	**238**	**292**	**0.066**	131	144	0.129
Co (mg kg^{-1})	0.090	0.141	0.325	0.101	0.142	0.286	0.022	0.072	0.253
Others									
Cr (mg kg^{-1})	10.5	5.15	0.112	5.17	5.59	0.721	**2.84**	**3.73**	**0.017**
Cd (mg kg^{-1})	0.064	0.000	0.220	0.005	0.022	0.144	0.011	0.044	0.292
Biomass (g plant^{-1})	9.17	14.4	0.419	13.0	13.1	0.938	19.0	19.5	0.886

Plants from northernmost Finland had the largest differences between infected and uninfected plants: the endophyte increased the concentrations of P, K, Ca, S, Fe, Zn, Cu and Al. Uninfected plants from Faroe Islands had greater concentration of Al and Fe than infected plants. In the Spanish plants, *Epichloë* endophyte increased the Cr concentration, a non-essential element for the plant.

Our results showed that *Epichloe* endophyte increased concentration of several elements in plants from Finland, but only Cr in plants from Spain. These results for Spanish plants differ from previous experiments with half-sib lines in which endophyte increased P and Zn content under field and greenhouse conditions (Zabalgogeazcoe *et al*., 2006; Vázquez de Aldana *et al*.,

2013). The greater nutrient content of E+ plants from Finland in Spanish conditions might indicate that nutrient allocation patterns are different for plants adapted to northern habitats compared to those growing in a Mediterranean habitat.

We found that plants from northern Europe (Faroe Islands and Finland) had, on average, a greater concentration of most mineral elements (P, K, Ca, Mg, S, Fe, Mn, Cu, Ni, Na, Al and Cr) than plants from southern Europe (Spain). Differences in Zn and Cd concentrations in plants from different origins were not statistically significant. Plants from Spain had greater biomass than northern-origin plants. Differences in nutrient concentrations may be affected by differences in the length and mean temperature of the growing season between original habitats. Thus, under more favourable climatic conditions (light and temperature), northern fescues adapted to short growing season and lower temperature may exhibit higher nutrient uptake and accumulation than southern fescues which are adapted to longer growing season and higher temperature.

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Soil carbon status of survived and restoring wood pasture in the protected area Natura 2000

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Abstract

Soils of survived and restoring wood pasture in the EU protected areas (Natura, 2000) were investigated. Soil samples were collected in 2012 from the 0–10, 10–20 and 20–30 cm depths. The study showed significantly different quantities of SOC and carbon forms accumulated in the soil. The soil of restoring wood pasture contained more organic carbon in 0–30 cm soil layer (24.0 g kg^{-1}) than in soil of the survived wood pasture (17.0 g kg^{-1}). The soils of protected areas differed in organic carbon stability. The largest amount of chemically ($C_{\text{chem. resist.}}$, C_{MHA}) and physically (C_{clay}) stabilized organic carbon forms, which represents the resistance to degradation and the possibility to sequestration, was established in the soil of restoring wood pasture. From an environmental viewpoint, the mentioned soil use is very important in order to accumulate carbon in the soil, to reduce the degradation of soil organic carbon and atmospheric CO_2 emission. The content and relative share of labile carbon (C_{H20}) in the soil of survived pasture was higher and increased with depth.

Keywords: restoring pasture, wood pasture, soil carbon, water soluble carbon, stabile carbon

Introduction

Forested pastures and wooded meadows are habitats that have significantly decreased or almost disappeared. Patches of these habitats, once numerous in Lithuania, have survived in the central part in moraine plain landscape. These types of habitat are a reminder of the very distant past: they existed even before the emergence of agriculture and livestock production and were supported by herds of large wild herbivores such as bison and tarpan. After World War II, wooded pastures disappeared rapidly because of the ban on grazing in forests and due to large-scale land reclamation. These wood pastures became overgrown with forest vegetation and the former diversity of the pasture vegetation declined. One of the tasks of nature protection is to preserve these pastures and to restore abandoned ones. The establishment of the Natura 2000 Network is one of the main actions undertaken at the European level to contribute to the maintenance of biodiversity (Bartula *et al.*, 2011). The main functions of the soil – biomass production and biodiversity, source of raw materials, storing, filtering and transforming nutrients, substances and water, physical and cultural environment for humans – are all directly dependent on the carbon pool. In line with new trends in global research, with increasingly more investigations and quantitative determinations of carbon flows being performed in order to reduce the negative impact of human activity on the environment, more attention in research has been given to carbon-compound transformations in agricultural soils (Liaudanskienė *et al.*, 2011). Agricultural grassland soils are comprehensively investigated in Lithuania, but there is no assessment of soil carbon composition in old wood pastures and in pastures under restoration. We hypothesize that differences in the amount and type of the organic matter inputs, and wood-pasture soil use affect the accumulation and stability of soil organic carbon.

Materials and methods

The soil *Endocalcari-Endohypergleyic Cambisol (CMg-n-h-can)* of the EU-protected area of wood pasture (Klampute) was investigated in this research. The soil of the survived wood pasture and of 'restoring wood pasture' (*i.e.* wood pasture that is under restoration) where old trees have been retained and shrubs were cut, was investigated. Soil samples were taken in 2012 from the 0–10, 10–20 and 20–30 cm depths with 6 boreholes per replicated plot. Three field replicates of wood pasture were investigated. For chemical analyses the soil samples were air-dried, visible roots and plant residues were manually removed, and the soil samples were then sieved through a 2-mm sieve and homogeneously mixed. For the analyses of SOC content and mobile humic acid fractions, an aliquot of the soil sample was passed through a 0.25-mm sieve. SOC content was determined by photometric procedure at the wavelength of 590 nm using the UV-VIS spectrophotometer (Cary 50; Varian, USA) after wet combustion according to Nikitin. Chemo-destructive fractionation was used for determination of stable (chemically resistant) carbon ($C_{chem\ resist.}$). Mobile humic acids (MHA) were extracted by 0.1M NaOH solution and determined according to Ponomariova and Potnikova. The data of chemical analyses were processed ($P < 0.05$) by the statistical program STAT ENG for EXCEL version 1.55. Each variable (n = 3) was displayed as mean ±standard error of the mean.

Results and discussion

Klampute is one of the areas (12 ha) where restoration of habitats is being carried out. The vegetation structure and species composition that are typical of Fennoscandian wood pastures (Mosquera–Losada *et al*., 2009) have survived in the southern part of the area. Single old oaks (*Quercus robur* L.) and large hawthorn (*Crataegus* spp.) shrubs, including intrusive herbaceous plant communities, grow there. The northern part of the area, formerly a Fennoscandian wood pasture, is overgrown with a dense forest. Species inherent to forest plant communities are entrenched in place of extinct meadow herbaceous plants. Evidence of the previously existing habitat can only be judged from the existence of isolated old trees (*Quercus robur* L., *Tilia cordata* Mill., *Fraxinus excelsior* L.). Tree crowns were matted, and growing stock closeness was 95% against the clearance. *Picea abies* (L.) H. Karst is quite widespread there, and this species is almost non-existent in the southern part of the area. Some shade and acidic soil-tolerant plants have been found, including *Oxalis acetosella* L., mosses *Eurhynchium angustirete* (Broth.) T. J. Kop., *Pleurozium schreberi* (Brid) Mitt. In 2011, restoration of the habitat in the Klampute area was launched. Young trees that were non-specific to the habitat were cut down during the winter. The grass was mown in 2012 and 2013. It is planned to acquire cattle, which will be grazed on the restored wood pastures in the future. Restoration success will depend on the rational use of these pastures.

The study showed significantly different quantity of SOC and carbon forms accumulated in the soil (Table 1).

Table 1. The soil variables of survived and restoring pasture in the protected area, 2012

Site description	Soil layer (cm)	SOC g kg⁻¹	C_{H20}	C_{H20} % from SOC	C chem. resist. g kg⁻¹	C chem. resist. from SOC	C_{MHA} g kg⁻¹	C_{clay}
Survived wood pasture	0–10	27.3±1.0	0.49±0.03	1.79	0.32±0.036	1.17	4.25±0.69	11.4±0.33
	10–20	16.8±1.1	0.34±0.02	2.02	0.11±0.004	0.65	2.30±0.68	7.64±0.50
	20–30	7.0±1.0	0.22±0.02	3.14	0.08±0.001	1.14	0.40±0.11	4.64±0.75
	0–30	17.0	0.35	2.32	0.17	0.99	2.32	7.89
Restoring wood pasture	0–10	34.3±5.9	0.54±0.04	1.57	0.30±0.016	4.58	6.31±0.62	17.6±0.80
	10–20	23.5±3.1	0.37±0.01	1.57	0.39±0.002	1.66	4.87±0.67	12.8±0.47
	20–30	14.2±1.2	0.26±0.01	1.83	0.25±0.046	1.76	2.74±0.59	7.29±0.41
	0–30	24.0	0.39	1.66	0.31	2.67	4.64	12.6

The soil of 'restoring wood pasture' contained more organic carbon (in the 0–30 cm soil layer): 24.0 g kg⁻¹ compared with 17.0 g kg⁻¹ in the soil of the surviving wood pasture. In the 0–10 cm soil layer of survived wood pasture, the most readily degradable water-soluble carbon accounted (1.79 g kg⁻¹) for a larger relative share of the SOC (2.32%). In restoring wood pasture overgrown by forest, more carbon was physically stabilized through binding with clay minerals (C_{clay}). Humification processes were also most intensive in the topsoil layer (0–10 cm), where the concentration of mobile humic acids (C_{MHA}) was the highest (6.31 g kg⁻¹) compared with that in the survived pasture (4.25 g kg⁻¹). The soils of the protected areas differed in organic carbon stability. All carbon indicators tested showed higher accumulation of SOC and chemically and physically stabilized carbon forms in the restoring wood pasture. One of the possible explanations for this is that the residues and roots of grasses decompose faster than residues and roots of broadleaf trees; this is likely due to the higher content of easily decomposable compounds found in grasses. Furthermore, the survived wood pasture was used, i.e. the grass has been mowed or grazed, and therefore there were less organic residues returned to the soil surface, whereas the re-naturalization was held in the restoring wood pasture. The soil of the survived wood pasture contained more labile, water-soluble carbon (C_{H20}), which shows its higher predisposition to transformation.

Conclusion

The largest amount of chemically and physically stabilized organic carbon forms, which represents the resistance to degradation and the possibility for sequestration, was established in the soil of the restoring wood pasture. The relative share of chemically resistant carbon ($C_{chem. resist}$) was higher in the soil of the restoring wood pasture and it decreased with depth. From an environmental viewpoint, the soil use is very important in order to accumulate carbon in the soil, to reduce the degradation of soil organic carbon and the net atmospheric CO_2 emission. Conversely, the content and relative share of labile carbon (C_{H20}) in the soil of the survived wood pasture was higher and it increased with depth.

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Plant succession and soil carbon sequestration potential of abandoned arable fields in a sub-humid Mediterranean environment

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Abstract

Abandonment of arable land is a major land use change in the Mediterranean region. The effect of this land use change on plant succession and on soil organic-carbon pools is an important factor for mitigating the increase of global greenhouse gases and climate change. The aim of this research was to quantify the soil carbon stocks of old fields along the secondary succession with different ages since abandonment (1-60 years) in a sub-humid Mediterranean climate of Northern Greece. The results revealed that soil is an important carbon sink of atmospheric CO_2, with mean average carbon sequestration of 2.68 t ha^{-1} and carbon sequestration rate 0.04 t ha^{-1} yr^{-1}. This function was closely related with plant succession characterized by an evolution from annual to perennial herbaceous functional groups while woody species appeared in later stages.

Keywords: soil carbon sink, plant functional groups, biomass, climate change

Introduction

Land use changes have significant effects on greenhouse gas emissions and carbon stocks in soil and vegetation (Feddema *et al.*, 2005). In most European countries, the transformed economies and social conditions of previous decades have resulted in significant land use changes leading to agricultural intensification, industrialization and migration of people from the rural areas (Alberti *et al.*, 2008). As a consequence, areas of marginal agriculture were abandoned, thus initiating secondary succession of the vegetation. This has occurred particularly in the Mediterranean region, and in Greece such abandonment is widespread in the mountainous areas (Papanastasis, 2007). Soil organic matter (SOM) can be a source or sink for atmospheric CO_2 depending on land use, management of soil, vegetation and water resources (Lal, 2009). The aim of this research was to quantify the current SOC stocks in old fields with different ages of abandonment.

Materials and methods

The research was conducted in Taxiarchis village located in the Holomontas mountain of Chalkidiki, North Greece. The climate is sub-humid Mediterranean. Seven fields were chosen (mean altitude 850 m), each reflecting a different period of abandonment of agricultural use, following a time series of ten years (abandonment age 1, 10, 20, 30, 40, 50 and 60 years each approximately). In each field, plant cover and species composition were measured along 5 transects, using the point method (Cook and Stubbendieck, 1986). Biomass was measured in 10 quadrats (1 x 1 m^2 each for woody species and 50 x 50 cm^2 each for herbaceous species). All biomass samples were oven dried at 60º C for 48 h. Soil samples were collected at a depth of 0-20cm. In the laboratory, soil total nitrogen and soil organic matter concentration were measured by the $K_2Cr_2O_7$ method using the modified Kjeldahl wet digestion procedure of Miller and Keeney (1982). The soil organic C content was estimated on a percentage basis and converted to tons per hectare using bulk density and soil depth. All data analyses were conducted using the software package SPSS 11.0. Significant differences for all statistical tests were evaluated at the level of $P \leq 0.05$.

Results and discussion

Agricultural abandonment resulted in spontaneous colonization of the old fields by various plant functional groups, with annual grasses, legumes and broadleaved herbs dominating in the early stages and their perennial counterparts replacing them in later stages, and with woody species appearing in older stages (Table 1). According to Bonet and Pausas (2004), the recovery of woody vegetation in Mediterranean old fields is usually hindered by herbaceous competition. Biomass tended to increase almost linearly along succession until 50 years, which is consistent with other studies on secondary succession in mesic Mediterranean conditions (La Mantia, 2008), but reduced from the age of 60 years.

Table 1. Mean average contribution (%) of functional groups and biomass (g m^{-2}) in fields with different ages since abandonment

Functional groups and biomass		Age of abandonment (years)						
		1	10	20	30	40	50	60
Grasses	Annual	33.51a	20.40c	26.90b	8.34d	0.56e	2.71de	0.73e
	Perennial	2.79e	6.96e	22.16d	46.78a	32.26c	46.19ab	38.55b
	Biennial	0.59b	0.38b	0.9b	0.12b	0	2.50a	0
Broadleaved herbs	Annual	27.84a	16.03b	17.94b	5.04c	1.44c	2.42c	2.39c
	Perennial	23.62b	46.80a	25.56b	25.53b	22.84b	17.59c	20.25b
Legumes	Annual	11.65a	9.43ab	6.54b	2.08bc	0.23c	2.18bc	2.47bc
	Perennial	0	0	0	6.20b	10.84a	2.69c	5.85b
Woody species	Shrubs	0	0	0	5.91b	6.08b	22.78a	6.56b
	Trees	0	0	0	0	0	0.94b	8.09a
Bracken fern		0	0	0	0	25.75a	0	15.11b
Biomass	Herbaceous	141.03bc	146.74bc	113.18c	222.86ab	261.37a	187.42b	53.84d
	Woody	0	0	0	24.43c	10.12d	438.18a	89.57b

Soil carbon dynamics after abandonment is connected to the development of the natural vegetation through secondary succession processes (Kosmas *et al.*, 2000). The high soil organic and nitrogen content found in the one-year-old field since abandonment perhaps indicates previous organic amendments (McLauchlan, 2006). Overall, organic carbon and nitrogen tended to increase from the early to the middle stage of abandonment, with a maximum reached at 30 years (Table 2).

Table 2. Mean average values (and standard deviations) of soil data in fields with different ages since abandonment

Soil data	Age of abandonment (years)						
	1	10	20	30	40	50	60
Bulk density (g cm^{-3})	0.22±0.03	0.27±0.04	0.20±0.02	0.25±0.05	0.23±0.02	0.28±0.03	0.27±0.04
Carbon (%)	2.43±0.56	1.51±0.42	3.08±0.77	2.96±0.47	2.06±0.28	1.96±0.66	2.13±0.47
Nitrogen(%)	0.22±0.04	0.13±0.03	0.27±0.08	0.32±0.09	0.22±0.03	0.17±0.06	0.14±0.05
C:N	11.29±1.08	12.05±1.15	11.39±0.84	9.41±2.84	9.58±1.89	11.52±4.19	15.22±4.4
Carbon (t ha^{-1})	2.2±0.64	2.4±0.90	2.37±0.33	3.48±0.59	3.10±0.88	2.05±0.57	2.69±0.62

This could be attributed to the increased presence in the cover of herbaceous plant functional groups, especially perennial grasses, which are a significant contributor of C in the soil (Santos *et al.*, 2011), while legumes usually increase the N availability due to N$_2$ fixation and higher N input via litter decomposition (Cuesta *et al.*, 2012). Thereafter, both carbon and nitrogen were reduced but remained stable until 60 years. This was maybe due to the increase of woody species in the vegetation composition, which contributed more biomass, as compared to herbaceous species (Table 1), but less soil organic carbon. According to Satti *et al.* (2003), species with slow growth rates such as Mediterranean woody species, exhibit high

concentrations of secondary compounds and chemical defences against herbivores, and have high lignin content which promotes slow decomposition (Castro *et al.*, 2010). This is also supported by the higher C:N ratio found in the field of 60 years since abandonment. Soil carbon storage (t ha^{-1}) followed the same trend, with carbon content showing a mean average value of 2.68 t ha^{-1} and a rate of 0.04 t ha^{-1} yr^{-1}. According to Cuesta *et al.* (2012) carbon accumulation in soils under secondary succession after crop abandonment is slow in Mediterranean environments; however, soil C storage is considered a viable option to mitigate climate change (Lal, 2004).

Conclusions

The results indicate that abandoned arable fields in a sub-humid Mediterranean environment are carbon sinks and that organic carbon storage in the soil is closely related to plant succession characterized by an evolution from annual to perennial herbaceous functional groups with woody species appearing in later stages.

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Theme 2 'Grasslands and ecosystem services'

Theme 2 invited papers

Functions of grassland and their potential in delivering ecosystem services

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Abstract

Grassland ecosystems provide ample services that are vital to nature and mankind. The role and the potential of grasslands to provide services has always been valued by farmers, it is now being increasingly recognized by the society as a whole. Services result from complex networks of interrelated ecosystem processes and both, processes and services, occur at a range of spatial and temporal scales. Generally, society expects that grasslands provide a range of services at the same time. This is difficult as often one intended utilization will dominate the others. The relationship of many of these services is therefore characterized by tradeoffs. Farmers play a centre in balancing conflicting relationships. One of the best options is to improve management in order to reduce tradeoffs. Research is increasingly needed to support innovative management in this respect. However, the mitigation of tradeoffs is in many cases difficult and limited and it requires an in-depth understanding of teh functional relationship between services. Balancing persistent tradeoffs is a matter of setting priorities which requires a discourse finally leading to an understanding among the various stakeholders. The practicability of the ecosystem services concept is most challenging both for research and development. The present review introduces conceptual ideas on the role of the ecosystem services concept in grassland farming. Major services and tradeoffs among services are identified. The feasibility to balance tradeoffs is analyzed in more detail in two case studies. One study is dealing with the various ways to provide protein to dairy cows and with the implications for other ecosystem services. The second example deals with wet grassland farming and the conservation of rare meadow birds.

Keywords: grassland, ecosystem function, biodiversity, water, climate, tradeoff

Introduction

The concept of ecosystem services has been developed to highlight the benefits of nature to mankind. De Groot (1987) stated that: nature provides regulation processes to maintain clean air, water and soil; it provides many resources (ranging from ore to wildlife products and genetic material) as well as space and a suitable substrate for many human activities (e.g. agriculture, recreation, *etc*.) and nature provides opportunities for reflection, aesthetic enjoyment and spiritual enrichment. De Groot (1987) hoped that the maintenance of environmental functions (goods and services) may serve as a unifying concept to provide a common long-term goal for both economists and conservationists and that the function-concept could be a useful instrument to merge ecological principles and economic procedures.
In 1997 a team of scientists and economists estimated the economic value of the world's natural ecosystems (Constanza *et al*., 1997). An early general review of ecosystem services by a range of experts in different topics was provided by Daily (1997) and further elaborated into a framework (Daily *et al*., 2000, Daily, 2000). Key issues of biodiversity, ecosystems stability, and ecosystems productivity were reviewed by Tilman (1999, 2000), Grime (1997), Chapin (2000), Kaiser (2000) and McCann (2000). The concept of tradeoffs, the reduction of one ecosystem service as a consequence of increased use of another, was described by Rodriguez *et al*., (2006) and was shown to be effective in pointing to ecological aspects of agricultural systems and to account for negative side effects of production (Power, 2010, Sanderson and Wätzold, 2010). In agricultural systems it is of interest i) how these systems depend on natural

ecosystems supporting them, ii) how producing goods (energy, protein, and fibre) and the supply of other services interact, and iii) how the identification and quantification of possible disservices and tradeoffs that are directly or indirectly related to production might affect the efficiency of production, affect other services of this system, and affect neighbouring natural ecosystems, water, soil and air.

The following review is concerned with agricultural grassland systems in Europe. Even though the review is focusing on one type of land-use only, namely grassland, the situation is highly complex. Grasslands are found under widely varying environmental conditions and their functioning is characterized by multiple interactions of environmental and management factors. The provisioning of certain services is thus difficult to predict. The situation is further complicated by the fact that functions and services are scale dependent. Services are valued by stakeholders. As it is most unlikely that all possible tradeoffs among different ecosystem services can be resolved there is a need to focus on those services that are considered by stakeholders to be most important, i.e. the grand challenges. Certainly, the identification of those challenges is not the same among the different stakeholders and the challenges may vary depending on the region and the status of the natural and agricultural resources. Therefore, in this review, general interests are preferentially considered compared to very particular interests. The perception of the challenges among stakeholders is a result of their intentions, be it to produce milk and meat from grassland or to conserve the diversity of the grassland-related biota. Based on intentions, the stakeholders are setting priorities.

The grand challenges, the intentions and the priorities will be used to set a framework for the analysis of ecosystem functions and services. After an introductory chapter on terms and basic concepts of ecosystem services we continue with describing major services from grasslands followed by an analysis of the potential to balance tradeoffs among services through adapted management. This procedure is illustrated in more detail in two case studies.

Terms, definitions and concepts of ecosystem services

In a report on the value of the world's ecosystem services and natural capital, Constanza *et al*. (1997) gave a set of useful definitions to distinguish ecosystem functions, goods and services. Ecosystem functions refer to the habitat, biological or system properties or processes of ecosystems, for example primary production, nutrient cycling, or biodiversity (Constanza *et al.*, 1997). Ecosystem goods and services represent the benefits humans derive, directly or indirectly, from ecosystem functions. Those benefits can be classified as provisioning services such as food and water, as regulating services that mitigate the effects of floods, droughts or land degradation, as supporting services such as soil formation or nutrient cycling and as cultural services that provide recreational, spiritual benefits (Millennium Ecosystem Assessment, 2005). Ecosystem services and functions usually do not show a one-to-one correspondence. They are not independent of one another and are often highly non-linear (Power, 2010).

Multi-criteria assessment techniques are commonly used when systems with multiple functions and services are to be analysed. Results are then presented in 'spider diagrams' with as many axes as services from the system (DeFries *et al.*, 2004). As an example, Figure 1 depicts different grassland farming situations with five main services with the production function being represented by axis 1. In a traditional pre-industrial grassland system (Figure 1A) only the production function was highly valued and intended, but low input and relatively low yields allowed ample provision of a range of other services. With the intensification of grassland farming (Figure 1B) the production increased markedly, almost approaching the site-specific potential, however at the expense of other services. Abandoning grasslands from agricultural use, which was a common phenomenon in many central and eastern European countries during the 1990s, ignores the production function completely (Figure 1C). While this may support

some functions and services, such as the stabilization of soils or the provision of clean water, it may be detrimental to other services such as biodiversity which has been shown to decrease with long term successional change (Kesting *et al*., 2009).
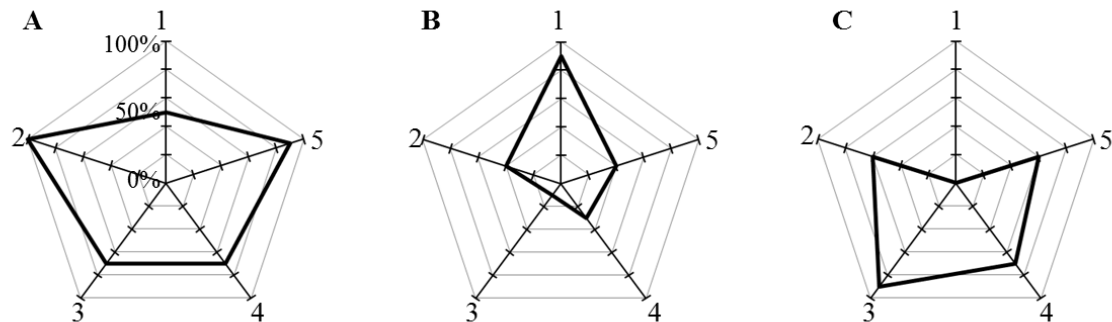
Figure 1. Provision of ecosystem services from grassland in (A) a pre-industrial situation, (B) current intensive farming and (C) a grassland abandoned from agricultural use, ecosystem services vary from 0 (no service/disservice) to 100% (maximum service possible under the particular conditions), schematic. 1: production service (forage, milk, meat), 2-5: other services, such as water, climate, diversity, culture).

In agricultural systems, humans make management choices which change the type, magnitude and relative mix of services provided by the system. These effects are termed tradeoffs and occur when the provision of one ecosystem service is reduced as a consequence of increased use of another service (Rodriguiz *et al*., 2006). Such tradeoffs are typically related to the production of goods as food, fibre and bioenergy, and affect regulating services as water purification, soil conservation, carbon storage or might result in direct disservices as water and air pollution by emissions (Power, 2010). The impact of tradeoffs can occur on a range of spatial and temporal scales: are the effects of the tradeoff felt locally, for example on-farm, or at a more distant location, does it occur instantly or later? Are the effects reversible and if so, how quickly can they be reversed (Power, 2010)?

Presenting results in 'spider diagrams' reflects a descriptive approach of assessing multiple ecosystem services. They do not provide in depth information on the functional relationships. Simply describing the magnitude of services from a production system falls to short. This does not provide us with the sound understanding of functional relationships needed for managing and balancing tradeoffs and disservices. However, relationships among different services are often characterized by multiple interactions, which are difficult to analyze. In order to explore the nature of relationships among services, a simple situation with only two services is depicted in Figure 1. The relationship of these services may be linear or non-linear, and it may be of a mutually exclusive, a non-interacting or mutually beneficial character (Figure 2). A typical example for the mutually exclusive type of relationship is the relation between production and nitrogen emission risk. A high grass or livestock production is usually achieved through a high input of nitrogen fertilizer. In general, the higher the fertilizer input the higher are nitrogen losses to the environment in form of denitrification, ammonia volatilization and/or nitrate leaching. However, ample research on the nitrogen use efficiency of grassland systems over the last decades has shown that there is - through better management - a considerable potential to minimize this tradeoff without compromising the production function. This is demonstrated by the dashed line of Figure 2A; the amount of service 1 is increased without change of the amount of service 2, and *vice versa*. The 'no-interaction' type of relationship (B) might be explained by the water retention function of grassland and grass production: as long as the grass sward is intact and thus the soil covered by a permanent vegetation, surface water run off will

be negligible, almost irrespective of the yield potential of the sward. Examples for mutually supportive functions (Figure 2C), i.e. a full win-win situation, are rare. Research over the last 15 years on sown, so-called artificial grasslands with a defined set of species in different mixtures, has revealed a considerable production benefit from increased phytodiversity. In temporary grassland (leys) a mixture of only a few grasses and legumes can be sufficient to yield much of this biodiversity effect (Lüscher *et al.*, 2008). However, older investigations on permanent grasslands do not confirm a mutually supportive relationship between phytodiversity and production, but rather suggest a humpback function of the D-type of Figure 2 (e.g. Oomes, 1992). More recent research on permanent grassland reports either no or even a negative relationship (Assaf *et al.*, 2011; Schneider *et al.*, 2011; Rose and Leuschner, 2012).
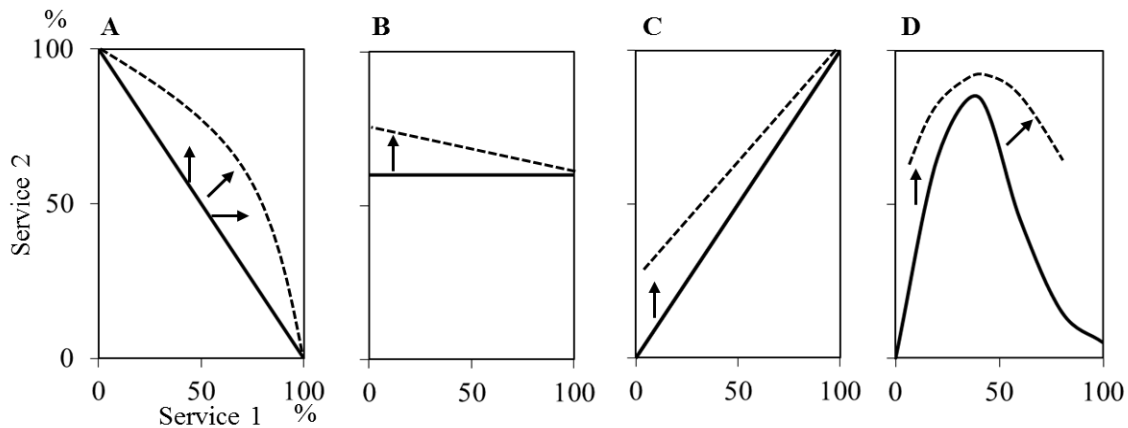
Figure 2. Schematic representation of the different types of functional relationships between two ecosystem services. Ecosystem services vary from 0 (no service/disservice) to 100% (maximum service possible under the particular conditions) provision. Kind of relationship of services: (A) linear mutually exclusive, (B) linear no interaction, (C) linear mutually supportive, (D) non-linear. Solid line: current status, dashed line: changed relationship, e.g. through improved management, with increased total service, see also text.

Grassland ecosystem services – The big five

Production

Grasslands are a main resource for agricultural production in Europe. According to the EU statistics they cover some 35% of Europe's utilized agricultural area and some 8% of the total European land surface (EUROSTAT, 2013). Recently, Peeters *et al.* (2014, this volume) suggested a new terminology of European grasslands. Grasslands are being defined as agricultural land that is covered mainly by grasses and the grass sward is either temporary or permanent, while permanent grassland has not been renewed for at least 10 years. The percentage of grassland of the utilized agricultural area in the different EU countries is highly variable and ranges from almost zero to almost 100% (Figure 3A). The reason why the grassland differs so much across the different countries is not readily identified. A comparison of agricultural land-use data did not reveal a clear effect of the size of the available area within a country. In general, permanent grassland sites in Europe can be found on agricultural land that is less suitable for arable farming. A main reason for this is often a surplus of water. This is confirmed by an obvious relationship between annual precipitation in a country and the percentage of permanent grassland of the utilized agricultural area (Figure 3B). The grassland area in Europe has been declining over the last decades. Between 1990 and 2006 the annual decline was -1.2%, albeit with a high variation between countries (EEA, 2010). Pressure on grassland habitats is still present. Grasslands have been converted mainly into afforested area but also into arable land. The conversion to arable land is particularly important on farms with intensive dairying and biogas production (mainly in Germany, Osterburg *et al.*, 2011).
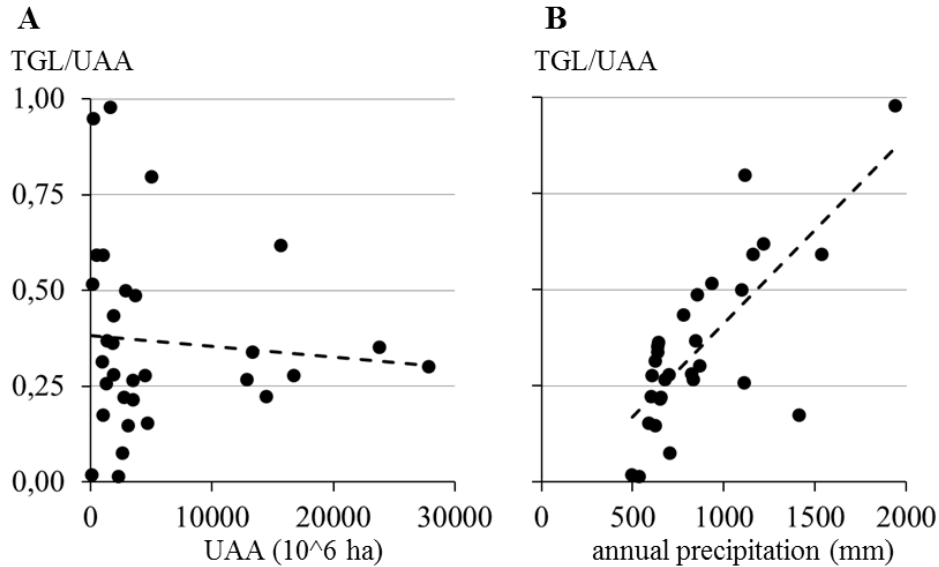
Figure 3. Ratio of the total grassland area (TGL) to the utilized agricultural area (UAA) in relation to (A) the UAA and (B) the average annual precipitation of the EU countries (Data source: EUROSTAT 2013, World Bank 2013, own calculations).

Grasslands are used for ruminant and equine husbandry and, in some countries, for the production of renewable energy. In Europe, they make up some 75% of the fodder area (grassland plus forage from arable land). The stocking rate of ruminants (cattle and sheep) is on average 1.2 LU (SD 0.7) per ha fodder area, and that of dairy cows is 0.5 (SD 0.3). The stocking rates of equines amount to 0.06 LU (SD 0.06) per ha total forage area and 0.07 (SD 0.08) per ha total grassland area (EUROSTAT, 2013). The production of milk and meat per total forage area varies greatly among countries and is positively related with the stocking rates. The more animals are kept per fodder area the higher is the output of milk and meat. On average, 2216 kg (SD 2075 kg) of milk and 227 kg of meat from ruminants (SD 589 kg) is produced per ha fodder area. Milk and meat yield per fodder area are positively related (Figure 4). This means that a lower milk production is not necessarily compensated by a higher meat production. The forage potential of the fodder areas is obviously highly variable among the different countries, so that in some countries a considerably high livestock performance of both dairy and beef/sheep is possible whereas in other countries it is not. To some extent this is confirmed by modeling data on the grassland productivity in Europe. Smit *et al.* (2008) estimated grassland productivity to vary between 10 and more than 70 dt/ha in the different environmental zones of Europe.

kg meat/ha fodder area
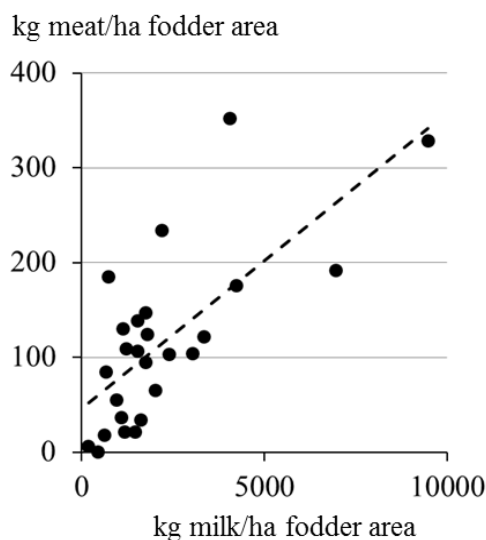
Figure 4. Production of milk per ha fodder area in relation to the production of meat (cattle, sheep) per ha fodder area in the EU countries (Data source: EUROSTAT 2013, own calculations).

Biodiversity

Grasslands in Europe harbour a huge biodiversity and they contribute most significantly to the overall diversity of the European agricultural landscapes. As an example, in Central Europe, almost one third of the total higher plant flora (i.e. some 1000 species) is related to grassland while the weed flora of the arable land only covers some 300 species (Ellenberg and Leuschner, 2010). The importance is also reflected by the fact that the grassland area is a major contributor to the high nature value farmland (EEA 2004). However, the biodiversity of grasslands is threatened and for several species groups (e.g., invertebrates, birds, amphibian, reptiles) decreasing trends could so far not be reversed; almost 50% of the grassland habitats are classified as being in an unfavourable conservation condition (EEA, 2013). A drastic example is that of grassland butterflies, as the referring diversity indicator has decreased by some 50% since 1990 (EEA 2013). There are several reasons of biodiversity losses, ranging from conversion of grasslands to arable land or woodland (including fallow land), amelioration of grassland sites through drainage or fertilization or intensification of grassland utilization. Halting losses has been given a high priority, not necessarily among farmers, but from the society perspective. There is an urgent need to make agricultural management more compatible with biodiversity concerns. The discussion is characterized by two main perspectives: (i) How can the diversity of grasslands be conserved and what is required from the agricultural management, and (ii) how can potential benefits from biodiverse systems be exploited for grassland farming. Strong management restrictions imposed on grasslands to maintain the nature conservation function has led to a decreasing interest of farmers in utilizing such land for livestock feeding and grasslands have been abandoned from agricultural use (Isselstein *et al.*, 2005). However, there is research available that has shown potential of integrating grasslands with a priority in nature conservation into up-to-date farming systems (e.g. Wrage *et al*., 2011; Jerrentrup *et al*., 2014). Yet, more research and development from the agricultural side is needed to identify and implement grassland farming systems and measures that make use of the grasslands without compromising the nature conservation value. Livestock husbandry systems as well as renewable energy production might be considered in this respect (Wachendorf and Soussana, 2012). Whether and to what extent biodiversity can support grass production is still debated. With regard to phytodiversity, model systems have shown that particularly under low input conditions diversity is enhancing productivity. This was mainly attributed to a higher niche complementarity and an increased resource-use efficiency of

diverse over monospecific swards (Hector *et al.*, 1999; Tilman *et al.*, 2001; Weigelt *et al.*, 2009). The biodiversity effect is specifically true at a low number of species, and the specific return of any extra species entering the sward is diminishing with a higher species number already being present (Hector *et al.*, 1999). The recently finished EU research project MULTISWARD could confirm that simple sown mixtures offer production advantages over monospecific swards (various authors, this volume). Those effects are obviously due to the functional composition of grass swards rather than to the mere species number (e.g. Grime,1997; Dias *et al.*, 2013). There are only very few examples available showing that a biodiversity effect would persist at a more intensive grassland management and at a higher level of manuring (e.g. Nyfeler *et al.*, 2009). For permanent grasslands, evidence of biodiversity revenues in terms of production is still scarce. There is some indication that with a more diverse botanical composition of the swards the feeding value might increase (Seither *et al.*, 2012).

Climate

Depending on site conditions and management, grasslands contribute to and mitigate greenhouse gas (GHG) emissions and thus play a key role in agriculture and climate change. A comprehensive review on the topic has been given by Conant (2010). There are three gases through which grassland soils take part in GHG fluxes. CO_2 is fixed as organic matter in the soil mainly through plant growth and the formation of transient or stable soil organic compounds and it is released through mineralization mainly after a disturbance event. N_2O emissions occur with an increased availability of mineral nitrogen in the soil. CH_4 may be released from wet grasslands on organic soils but the indirect emission through grass-fed ruminants is more important (Conant, 2010). In general, agriculture is the largest single contributor to N_2O and CH_4 emissions to the atmosphere and correspondingly agriculture is expected to reduce emissions. Due to the complex nature of GHG fluxes between soil and atmosphere and the different ways, either direct or indirect via livestock, how grasslands account for emissions, mitigation strategies are not straightforward and single measures may reduce one emission pathway but at the same time may increase another one (Flessa *et al.*, 2012).

The amount of carbon sequestration to the soil depends on the grassland management and the soil water status. As a rule, the higher the soil water table the higher the carbon sequestration to the soil or - on organic soils - the lower the carbon release via mineralization of organic matter (Flessa *et al.*, 2012). Maintaining and re-establishing permanent grasslands on organic soils rather than conversion to arable land is a most effective way of maintaining and increasing soil carbon content. It had been assessed that conversion of grassland to arable land would reduce the organic carbon content in the top soil layer (0-30 cm) by 30 to 36% in the long run (Flessa *et al.*, 2012). Increasing grassland productivity through improved management has a potential to increase soil organic carbon (Conant, 2010) although the processes are not fully understood. Fornara *et al.* (2013) did not find an increased carbon content of grassland soils that had been fertilized with a balanced nutrient mix over 19 years as compared to an unfertilized control treatment. However, an unbalanced fertilization with nitrogen only increased soil organic carbon. It was assumed that nitrogen fertilization increased root growth. Increasing productivity by higher fertilization bears the risk of higher N_2O emissions, thus counteracting benefits in carbon storage. N_2O emissions are closely related to the nitrogen surplus both at farm and field level balances, which was shown among others by the EU Dairyman project (Aarts *et al.*, 2013). A high nitrogen surplus is either due to heavy nitrogen fertilization or to high stocking rates that come along with high amounts of purchased concentrate and protein feed.

Water

Grasslands affect local and regional water cycles (Ehlers and Goss, 2003). Compared to woodlands, grasslands have a considerably higher groundwater recharge as water fluxes from the soil to the atmosphere through interception water are lower (Ehlers and Goss, 2003). The quality of leaching water is often higher under grassland as compared to arable land because of a lower mineral, in particular nitrate, load of the soil water. In temperate climates unproductive evaporational water losses are generally lower than transpirational ones because the soil surface is covered by grass vegetation and thus less exposed to evaporation. The amount of water transpired by the vegetation is directly related to production (de Wit, 1958). Increasing production, e.g. by fertilization, therefore increases water consumption by the grass vegetation (Husemann and Wesche, 1964); yet the water-use efficiency may rise due to a decreasing evaporation to transpiration ratio (Ehlers and Goss, 2003). In a recent publication on semi-natural grassland, Rose *et al*. (2011) confirmed that fertilization increases production and transpiration and that this had a clear decreasing effect on the amount of groundwater recharge. Thus, the way grassland is managed has an impact on the soil water table and the landscape hydrology (Rose *et al*., 2011). A strong feature of grasslands is the water retention capacity. Well managed grass swards are most effective in protecting soils from water surface runoff and soil erosion. In hilly regions where the soils are prone to surface water runoff the percentage of grasslands in the land use is important for the likelyhood of river floodings. In a model approach, van der Ploeg *et al*. (1999) calculated an increasing risk of floodings of the river Rhine at Cologne as a result of a loss of permanent grassland through conversion to arable land by 6% over a period of 20 years. They concluded that the maintenance of grasslands should be aimed at in order to decrease the risks of floodings of the large rivers.

Culture

Grasslands provide aesthetic values, opportunities for recreation and nature education and are a part of an open preferably diversified landscape (Hönigová *et al*., 2012). Mostly, this applies not only to natural or semi-natural grasslands but to managed grassland as well, while the degree of the socio-cultural service decreases with intensity and loss of biodiversity (Lindemann-Matthies, 2010). Some aspects and implications related to agricultural production, environment and landscape have been extensively reviewed by Gibon (2005) and Quetier *et al*. (2010) among others. To assess the non-material benefits of people from cultural ecosystem services and how they might be affected by changes in other services, that could be an increase or decrease in the intensity of agricultural management resulting in an altered landscape, is probably the most difficult task. Information on this topic and especially on possible tradeoffs is comparably scarce. In the generally positive perception of grazed grassland, aspects of landscape, aesthetic values, biodiversity and concern for animal welfare are combined (Van den Pol-van Dasselaar, 2008). This can have economic consequences for farmers and consumers when pasture-grazed dairy products are put on the market that are either promoted or ask for higher prices.

The challenge of balancing tradeoffs - Two case studies

Balancing tradeoffs among ecosystem services sets an important framework for grassland management-related research of today as ecosystem services from grassland are more and more considered as being relevant for society (Hopkins and Wilkins, 2006). Tradeoffs are looked at from various viewpoints and with different foci. Sanderson and Wätzold (2010) in their review on ecosystem services point to a range of pressing issues, e.g. the stocking rate-livestock tradeoff, the forage quantity-quality dilemma, the production-carbon storage relationship or the economic consequences of balancing tradeoffs. They conclude that any new recommendations of policies, programmes or payment schemes must acknowledge and understand the related

tradeoffs and their interconnections – and that designing cost-effective policies is an important way to mitigate tradeoffs and use resources efficiently. In the following, two case studies are introduced to point at two quite different aspects of grassland management: firstly, intensive grassland management for provision of and protein (and energy) in dairy farming and, secondly, extensive grassland as a habitat for meadow bird breeding.

Protein for milk production and grassland management

Nitrogen is a key factor in grassland farming and in ruminant nutrition and through the provision of feed from grasslands these two compartments are closely related (for a recent comprehensive overview on this see Taube *et al.*, 2014). Protein is often a limited resource on farms with high yielding dairy cows. Many farmers perceive grass protein as not fully meeting the dietary requirements of high performing dairy and rely on in-bought protein feed. These protein sources are of high quality and they yield extra milk. However, a nitrogen balance of the dairy ration often shows that the mere amount of nitrogen provided with the roughage was sufficient. Depending on how much protein is purchased and where it had been produced, ecosystem services from agricultural land are concerned. For example, if soyabean from overseas is used ecosystem services from the farmland where soyabean is fed as well as services from the arable land where the soyabean had been produced are affected. The latter being an example for a direct dependency of services at a global scale. Obviously, there are economic benefits for global business partners which can be explained by the comparative advantage of production and trade. However, there are also obvious tradeoffs of this practice in terms of emission risks (e.g. greenhouse gas mitigation on the dairy farm) or biodiversity conservation (e.g. losses of natural diversity where the feed is produced). Del Prado *et al.* (2013), using practical farm data in a modelling approach, state that dairy diet is a strong factor explaining differences in GHG emissions from milk production and that the amount of feed that could have been utilized for human nutrition is a good predictor of the carbon footprint. The purchase of concentrate and protein feed has long been shown to boost nitrogen surpluses of nutrient balances of dairy farms (e.g. Aarts *et al.*, 1992, as one of the early papers) with the result that the grass and arable fields are burdened with heavy fertilization. Thus, there is a tradeoff between milk production (via increased purchased feed) and the nitrogen emission risk of dairy farming. However, this tradeoff between milk production and nitrogen efficiency is not a fully exclusive one as is shown with the solid line in Figure 2A. If nitrogen in the roughage is limited, feeding additional protein will increase the overall nitrogen efficiency because it improves the conversion of the energy contained in the roughage into milk product. In addition, this will also reduce the land-use for producing the dairy feed (Yan *et al.*, 2013a). The tradeoff may also be diluted by improved techniques of manure treatment on the farm. These measures will increase production through increased efficiency of carbon and nitrogen fluxes in the soil – grass – ruminant system and the relationship between the services will rather follow the dashed line of Figure 2A. The extent of the global tradeoff between the production service of the dairy and the biodiversity service overseas is dependent on the land type that is allocated to grow soyabean, e.g. whether it is land that had recently been converted from tropical forest and extensively managed rangelands or it is established cropland. In order to encounter the global tradeoff it has been suggested to increase the use of domestic protein sources (Taube *et al.*, 2014). In this respect, grasslands and grass clover leys have a huge potential as protein yields per ha grass exceed those of grain crops by far (Taube *et al.*, 2014).

In general, there are two strategies to increase the protein yield from grassland. The first strategy includes an increased nitrogen fertilization or fostering the growth of forage legumes (i.e. grass-clover systems). Clover-based systems without using additional mineral fertilizer have a clear advantage in terms of nitrogen emission risks, albeit with somewhat reduced grass yields. Compared to grass-nitrogen systems, losses via denitrification (N_2O) and leaching

(NO₃) are lower, i.e. there are fewer disservices with regard to GHG releases (Yan *et al.*, 2013b) and ground water contamination with nitrate (Eriksen *et al.*, 2010). The difference between grass-nitrogen and clover-based systems is likely to decrease with increasing stocking rates (Andrews *et al.*, 2007), in particular when high stocking rates are based on bought-in feed. On dairy farms, nitrogen is circulating in great quantities in the system while only small amounts are released from the system via milk and meat (Whitehead, 1995) The remaining nitrogen has to be recycled in the soil-plant system. The higher the stocking rates and the higher the amount of milk being produced per ha, the larger the nitrogen flux.

Grassland-based livestock production ranges from extensive, all-year grazing on permanent grassland to intensive housing in stables (indoor keeping) based on cut grassland, leys, maize and concentrates. Pathways for losses of N by leaching and gaseous emissions, mainly NH_3 and N_2O differ among systems. With housed livestock, dairy especially, and application of manures, NH_3 losses can be substantial while N leaching is usually relatively small (Benke, 1992; Anger, 2001). Cuttle and Jarvis (2005) report larger NH_3 losses from housing and manure storage than from application. Consequently, efficiency of N cycling can be mainly improved by technical solutions in reducing NH_3 losses in storing and applying manures (Anger and Kühbauch, 1999). In grazed systems N leaching losses, mainly from high N inputs at urine spots, are almost inevitable. The extent of N leaching is related to the number and timing of expected urine spots, feeding of concentrates, and N input from mineral fertilizers and manure (White *et al.*, 2001; Pleasants *et al.*, 2007; Moir *et al.*, 2011). Apart from plant uptake and N leaching, NH_3 losses, denitrification and microbial immobilization are other sources or sinks for excreted N.

The later in the grazing season, the higher the stocking rate and the additional mineral and organic fertilization with nitrogen, the higher the general total load of the system with N leaching (Benke, 1992; Wachendorf *et al.*, 2006; Kayser *et al.*, 2008). Gaseous losses in the form of N_2O are usually higher under grazing than in cut grassland if conditions are the same otherwise (Oenema *et al.*, 1997). Generally, and different than in cut systems, reduction in N losses in grazing systems can be achieved more by adapting and adjusting the management. By introducing a more reduced and temporally adjusted N-fertilization and a combination of cutting and grazing - temporal succession and changes in utilization - both, nitrate leaching and the formation of N_2O might be reduced (Anger, 2001). In conclusion, at the farm scale nutrient efficiency is thus depending on how well the cycling of nutrients within the soil-plant-ruminant system is organized. Losses reduce the cost-effectiveness (profitability) of production, and, as emissions, contribute to environmental stress. The extent to what losses occur is to a great deal depending on management factors.

Meadow birds and grassland management

Lowland fens are important for biodiversity and for providing a range of other ecosystem services among which are landscape hydrology and carbon mitigation (Kratz and Pfadenhauer, 2001; Bragg and Lindsay, 2003). As a result of the intensification of agriculture pristine lowland fens are drastically reduced worldwide (Parish *et al.*, 2008). Open landscape lowland grasslands are especially important as breeding habitats for meadow birds. Besides preservation of organic carbon in soil, landscape hydrology and aesthetics, reestablishment of specific plant communities providing a habitat for meadow-breeding birds is often a priority target of restoration and conservation of fen areas. Restoration measures commonly include rewetting, drastic restrictions on fertilizer use or typically a call for complete de-eutrophication and use as extensive grasslands and a later first cutting date or start of grazing (Klimkowska *et al.*, 2010; Müller *et al.*, 2010). Given this situation, it is obvious that the production function of grasslands suffer. Yields successively decrease with time, more so will the feeding value of the forage. Stocking rates and defoliation frequencies are low, the grazing period is limited due

to wet soils. Livestock performance from wet grasslands is therefore low and there is a definite priority of the environmental services. However, also within this production system there is an incentive to reduce the tradeoff between the production and the environmental function. If the grasslands would be abandoned from agricultural use, the other services would be at risk. Meadow-breeding birds require a habitat quality with a heterogeneous vegetation structure composed of sufficiently high proportions of open, short-grass areas in combination with patches of taller vegetation (Fondell and Ball, 2004; Verhulst, 2011). Grazing intensity determines defoliation intensity, soil compaction, local nutrient returns in the form of excreta, and avoidance, and is thus a key management variable influencing the structure and composition of pastures, i.e. patch type, scale and stability (Pavlu *et al.*, 2006; Dumont *et al.*, 2011). At a restricted stocking density usually undergrazing and selective grazing occur (Adler *et al.*, 2001) creating patches of different sward height at different spatial scales and with a comparatively high stability (Rook *et al.*, 2004, Tallowin *et al.*, 2005). While providing an adequate habitat for meadow birds, enhancing biodiversity and ensuring soil protection agricultural use with heifers, oxen or suckler cows can only be economically viable if costs for maintenance are kept to a minimum (Mills *et al.*, 2007). Due to selective grazing, animals might select diets of a better quality than the mean of the herbage on offer (Phillips, 2002; Rook *et al.*, 2004). Therefore, with reduced stocking, even less-productive grassland might be used for efficient livestock farming (Isselstein *et al.*, 2007). In investigations on extensive grazing with oxen on fen grassland in northwest Germany, Benke and Isselstein (2001) found relatively high individual daily live weight gains of 418–871 g head-1 with an average of 699 g head-1 during 1993–2000. The potential gross biomass growth was about 80 GJ NEL ha-1, while the net pasture performance amounted to 14.3 GJ NEL ha^{-1} in 1999 and 21.3 GJ NEL ha^{-1} in 2000. Thus, the grass leavings of about 80% in 1999 and 73% in 2000 were very high (Wrage *et al.*, 2011). However, breeding success of meadow birds is also directly related to stocking rate. Müller *et al.* (2009) found a positive linear relationship between stocking rate and number of nests lost by trampling. True nest survival was reduced from 60% at a stocking rate of 1.5-3 LU ha^{-1} to 20% at 4.5 LU ha^{-1}. Scale issues, e.g. the size of the field and the resulting influence of animal behaviour on habitat pattern, seemed to play a role, too. They conclude that up to a density of 2 LU ha^{-1} trampling losses of nests are still moderate and can be tolerated because of the positive effects of grazing on sward structure and nesting habitat (Müller *et al.*, 2009).

We might summarize as follows: the intention to protect meadow birds and in turn preserve and provide a suitable habitat for breeding and rearing can be seen as the main driver, and target ecosystem service as well, that determines grassland management in designated areas. As explained above this management entails extensive grazing and generally facilitates other connected ecosystem services as soil protection, biodiversity, recreation *etc*. Limited agricultural use (production of goods as an ecosystem service), reduced biomass and greatly varying forage quality can be identified as the main tradeoffs. Tradeoffs at the farm level might occur locally distant when other fields have to be farmed more intensively, with possible risks for groundwater and air pollution, to make up for the restricted use in the fen area. In time, other implications directly related to the restrictions of grassland management and nutrient input are imaginable which might be adversely affecting the botanical composition (Janssens *et al.*, 1998) and forage quality for grazers but also the abundance of soil fauna as a feed source for meadow birds (Altenburg and Wymenga, 1998; Müller *et al.*, 2010). For example, even if managed extensively, K leaching from fen grassland can be higher than input with rain, ground- and surface water (Koppisch *et al.*, 2001). Finally, a nutrient imbalance occurs between N, P and K, as N is still supplied by the mineralization of peat, while K becomes deficient (Pfadenhauer *et al.*, 2001; Kayser and Isselstein, 2005).

Outlook: future developments and research needs

The awareness that grasslands are multi-functional and provide a range of highly valued services in addition to the production function has been steadily increasing over the last two decades. European agricultural policy has responded to this perception and is placing more emphasis on the environmental functions of grasslands. The concept of ecosystem services could be used as a framework in which well-justified solutions for a grassland management that support a range of functions are developed. This is becoming even more important with the recently claimed requirement for 'sustainable intensification' which appears as the new paradigm of agriculture. While the concept of ecosystem services provides a valuable guiding principle and helps to better understand the tradeoffs among different services, its practicability is also limited. A major reason for this limitation is the high complexity of interacting processes that determine functions and services which make the concept difficult to apply. A common experience in this respect is that a deliberate change of one function of the system results in unexpected disfunctions and disservices in other parts. To approach such problems more research and development is required on the nature of relationships among different functions employing more complex experimental designs. Irrespective of the research requirements the concept of ecosystem services is already supporting the societal discourse on where to go and as a result of this, the intentions and priorities of stakeholders are becoming obvious. As an example, within the EU Dairyman project farmers had been offered a selection of management options to improve their farming situation in terms of several sustainability measures (Aarts *et al.*, 2013). The response of farmers was documented. It could be shown that the farmers differ widely in their preferences for actions. These differences occurred mainly among different regions in Northwest Europe reflecting the wide variation in the physical and socio-economic conditions in the different regions. Likewise, if other stakeholders were asked to express their preferences, a broad variation would have appeared. As has been shown above, the clarity on intentions and preferences of stakeholders is a prerequisite to balance tradeoffs.

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Comparing synthetic and natural grasslands for agricultural production and ecosystem service

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Abstract

Whilst the concept of ecosystem service is relatively new, the importance and benefits of natural grasslands to the environment has been long established. These complex bio-diverse ecosystems, as well as sustaining rich communities of flora and fauna, provide a range of environmental benefits including water, nutrient, and carbon capture. However, the perpetuity of natural grasslands and their associated benefits are under increased threat from pressures to feed and house our increasing population, urban expansion, and also through climate change. Regular ploughing and resowing of grasslands has led to soil erosion, depletion of scarce nutrient resources, pollution of our waterways, and releases of harmful greenhouse gases, the latter in particular exacerbated by livestock agriculture. A response is necessary both to reduce negative impacts of agriculture on the environment and, wherever possible, to engineer a positive ecosystem service. The genomic and phenomic diversity available in grass and clover species, and further access to novel variants through hybridization with wild-type relatives with suitable technologies to assist in their selection, provide alternatives to current plant varieties and increased capacity for efficient and 'climate-smart' agricultural practice. Holistic approaches to plant breeding can produce varieties that both safeguard agricultural production and provide some valuable ecosystem service.

Keywords: *Festulolium*, clover, semi-natural grasslands, grassland diversity, ecosystem services

Introduction

Among diverse grassland ecosystems, it is necessary to distinguish climatically determined grasslands, where water availability is insufficient for development of forest ecosystems and where natural vegetation remains in dynamic equilibrium with herbivores (Lauenroth, 1979), from the anthropogenically generated grasslands, located mainly within temperate climate regions, where woody vegetation is excluded and herbaceous plant communities maintained by appropriate human intervention and by livestock agriculture. It is possible to further divide the latter grassland type into long-term naturalized grasslands and those that are cultivated, which differ according to level of intensification. However, all grasslands are multifunctional and to different degrees are capable of playing important roles in agronomic, economic and social activities. Whilst an increase in grassland production in terms of its provision as safe, healthy and economically sustainable fodder for livestock consumption was viewed as an important priority for national security, and still remains a priority, it is also important to acknowledge the important role of grassland ecosystems in all our lives, from the air we breathe to the water we drink, and wherever possible, manage grassland ecosystems to maximize their potential to deliver environmental benefits. The concept of 'ecosystem services' was outlined within the Millennium Ecosystem Assessment (2005) (http://www.maweb.org/en/index.aspx). A recent review of 17 grassland biodiversity experiments revealed that 84% of 147 grassland

species displayed ecosystem functions, as measured, at least once (Isbell *et al.*, 2011), and while species may appear redundant under one circumstance, they may otherwise function fully in another, leading to the use of species mixtures to help ensure the expression of multiple ecosystem services, particularly when challenged by changing environmental conditions. Similarly for grassland agricultural production, it is normal practice both in the UK and in Ireland to re-sow grasslands with variety mixtures to encourage resilience to predictable stresses, long growing seasons and ultimately sustainable yields.

Whilst the 'market value' of grasslands to agriculture is obvious through the production of meat, wool and milk from grazing animals, they are being increasingly recognized at the global scale for their non-market contribution to carbon sequestration, prevention of soil erosion and for genetic conservation. Other more subtle ecosystem-supporting services include pollination processes, improved soil structure, decreased nutrient leaching and nitrogen fixation. Ecosystem services may be produced *in situ*, e.g. through biomass production and carbon sequestration, or adjacent to other habitats and organisms, e.g. preventing leaching of nutrients downhill into rivers, streams or wetlands, or a source of flowers for pollinators from other locations.

Many grasslands are 'highly improved' and as a consequence have significantly reduced ecosystem services compared to semi-natural or natural grassland ecosystems. The plight of semi-natural and natural grasslands is well known, with a loss of at least 98% of our wild flower meadows in Britain over the last 50 years (Peterken, 2013). Their fragmentation and degradation have reduced their potential for ecosystem services significantly.

For grasslands, as with forest ecosystems, plant-soil interactions involve different and complex cycling elements, particularly of carbon (C), nitrogen (N), and phosphorus (P). These operate (i) in plants, where N, P and C are combined in organic matter synthesis, accumulation and long-term sequestration in soils; and (ii) in soils, where microbes feed abundantly on C and recapture and recycle mineral N and P. In most circumstances, grasslands can sequester C, N and P for relatively long-term periods, so contributing to the atmospheric CO_2 sink, and reducing release of N compounds into water and atmosphere and their associated environmental risks. Grasslands are land-use systems that can be very favourable for environmental preservation. However, this idealistic view has to be tempered, as livestock decouple C and N–P cycles, through their urine and faeces depositions and their methane (CH_4) emissions, offsetting, sometimes to a significant extent, the beneficial effect of grassland vegetation–soil interactions.

Alternative viewpoints persist between ecologists and crop geneticists as to what extent it is possible to reduce the detrimental impacts of grassland agriculture on the environment by combined use of best farming practice and the inclusion of new and more sustainable grassland crop varieties. Entrenched viewpoints are sustained by some crop geneticists' naïve understanding of ecosystem complexity and certain field ecologists' ignorance of the potential of new plant breeding technologies, as well as by others whose priorities, for whatever reason, may differ significantly. Whilst risking being considered naïve, an argument is presented herein where a 'win-win' scenario might be achieved, but, as authors with different viewpoints ourselves, we counter-balance the argument by specific reference to the greater service diversity obtained from semi-natural grasslands, both by their species diversity and the far more stable soil system they support.

Through the incorporation of new and appropriate technologies and germplasm, it is proposed on the one hand that there are significant opportunities available to 'untap' potential for ecosystem service from novel grasslands, whilst also providing productive crops considered fit for agricultural purpose. However, before these are outlined it should be clarified that the authors consider any new option for grassland agriculture that might safeguard the needs of both agriculture and the environment, will not in any way displace current priorities to

safeguard existing semi-natural grassland ecosystems, whose complex and diverse benefits are not likely to be easily reproduced.

Semi-natural grasslands and their ecosystem services

The value of semi-natural grasslands for ecosystem service should never be underestimated. To paraphrase the British Ecological Society Bulletin Bogbean Bennet Cartoon-strip (http://homepages.abdn.ac.uk/g.j.pierce/pages/bbb.htm), any attempt to compensate for the destruction of a habitat by reproducing something similar elsewhere is like 'moving the Mona Lisa' by scraping away all the paint on the canvas and reconstructing it elsewhere: the paint may be the same, but the interconnectivity of the paint flakes is totally lost. Ecosystems are highly complex. For the most part we study only certain aspects such as carbon sequestration, productivity, or species composition; be it birds, mammals, invertebrates or plants. The soil may be studied for respiration (productivity), arthropods, or fungi, but the full ecosystem *in toto* is virtually impossible to describe and quantify. So the ability to recreate it is not, in reality, a feasible option.

In Ireland, as in Britain, grasslands are a derived habitat, maintained by farming practice, either by grazing or cutting. The last 50 years or more have seen widespread intensification, largely driven by EU policies and incentives (Hickie *et al.,* 1999). Grasslands were traditionally managed by low-intensity farming and semi-natural grasslands differed in species composition as a consequence of, e.g., soil pH or water content (O'Sullivan, 1982). The EU drive for productivity beginning in the 1970s led to the ploughing up of grasslands for rotation crops and reseeding for silage, with a concomitant reliance on chemicals, including fertilizer application. These changes resulted in the reduction of semi-natural grassland and therefore plant species diversity in a grassland landscape. In particular, the traditional extensive practice of cutting meadows for hay has declined substantially in recent decades (Peterken, 2013). Semi-natural grasslands have thus imperceptibly become very rare in Ireland, Britain and mainland Europe (Fuller, 1987; Baldock, 1989; Feehan, 2003; Sullivan *et al.*, 2010). Their conservation is now considered a priority and forms part of EU-, as well as national policy; the EU Habitats Directive lists 31 semi-natural grassland habitats in Europe, and national programmes are focused on conserving the range of these habitats that still remain (Critchley *et al.,* 2003; EEA, 2004; Stevens *et al.*, 2010; O'Neill *et al.*, 2013).

Aside from the ethics of conserving increasingly rare species-rich habitats, it is now seen as important to quantify their contribution to the environment as ecosystem services. This relatively recent concept relates to the current need to put an economic value on our environment, since, if it affects us economically, it may induce governments to take action. The drive to be productive and increase profit margins is influenced in this way, but the economic losses resulting from agricultural intensification also require evaluation (Purvis *et al.,* 2008; Dreschler *et al.,* 2010).

Individual plant traits in grassland studies have come to the fore in relation to ecosystem services from semi-natural and natural grasslands in Europe. A Europe-wide study named VESTA (Garnier *et al*., 2007) involving 11 European sites illustrates that experiments that can isolate direct effects of climate and land use from indirect effects, such as changes in community functional composition, advance our understanding of the role of plant traits as linkages between environmental change and ecosystem properties. Inclusion of environmental variables such as climate, soil, and disturbance in quantitative analyses makes it possible to test hypotheses about the pathways that determine ecosystem properties through modification of plant traits in communities.

Value and productivity of species-rich swards

Arguments for intensive highly-productive agriculture often overlook the fact that grasslands rich in biodiversity have been shown to be more productive than species-poor swards (Tilman *et al.*, 1996; 1997), especially grasslands with high forb diversity and resultant complementarities of resource use (Hooper *et al.*, 2005; Bullock *et al.*, 2007). The number of legumes alone does not account for a yield increase; instead the wide range of temporal and spatial growth patterns exhibited by different forbs maximizes the use of resources and can increase fodder value (Hofmann and Isselstein, 2005). Forb diversity is a key factor in the provision of nutritious hay, particularly for horses that do not eat silage (Allison and Day, 1999). The high quality of the species-rich grasslands of the Burren is widely known and farmers from the Irish midlands move animals onto the High Burren to increase bone and muscle quality before fattening on richer soils in the spring (Dunford, 2002; Williams *et al.*, 2009).

The experimental addition of species number on prairie grassland experiencing a fluctuating climate created greater below-ground biomass and temporal stability that, in turn, enhanced ecosystem services such as fodder provision and biofuel production (Tilman *et al.,* 2006a). Over ten years the experimental manipulation of native grassland perennial mixtures (Low Input High Diversity Grasslands, LIHD), of varied species number, demonstrated that these required much less pesticide and fertilizer applications than conventional monoculture crops such as corn and soybean, so were less costly to produce in terms of fossil energy inputs (Tilman *et al.*, 2006b). Carbon sequestration in the grassland soils was very high, whereas that of the annual crops was negligible, and would remain so over time. The more diverse the grasslands, the more biomass they produced. As a result, high-diversity grasslands had increasingly higher bioenergy yields that were 238% greater than monoculture yields after a decade (Tilman *et al.*, 2006b). The LIHDs were also more beneficial in that they could be grown on degraded soils where no monocultures could easily be grown.

Using an agro-ecosystem approach, the full benefits of a balance between crops, pests and their predators can be evaluated (Altieri, 2008), in contrast to the relative efficiency of a more industrialized approach to agriculture, where the real costs of fuels and chemical application need to be factored in, as well as the relative pest vulnerability of monotypic crops, including intensively farmed grasslands. The advantages to the environment of organic farming must not be discounted (Niggli *et al.*, 2007), especially as organic farming yields are often no less than those of non-organic farming, contrary to widely-held belief (Badgley *et al.*, 2007). On the other hand, in relation to ecosystem services it is important to address the range of these that biodiversity can offer, including habitat, as well as species (flora and fauna) diversity (Council of the European Union, 2010).

Pollinator services

Forbs in a grassland sward are important, as these are predominantly insect-pollinated species (as opposed to wind-pollinated grasses) and therefore they provide both pollen and nectar for a range of dipteran and other invertebrates (Williams,1988; Branquart and Hemptinne, 2000; Peterken, 2013). These in turn provide essential pollination for crops, notably oilseed rape, but also fruit, in particular nectar-producing trees such as apple and pear (Free, 1993). The recent decline in bees, both the honey bee due to diseases, and bumble and solitary bees, has caused much concern (Williams, 1982; Williams *et al.*, 1991; Carvell, 2002). This has been attributed in large part to loss and fragmentation of semi-natural habitat with services not available in arable fields (Carvell *et al.*, 2006). The reduction of a sustained source of food for these species is likely to be a factor in their decline, since once early-flowering crops such as oilseed rape finish flowering, there must be a series of other species to provide food until the late autumn (Prŷs-Jones and Corbet, 1991; Carvell, 2002). The type of plant species also affects the

pollinator species, since early-flowering annuals provide less nectar and may be more suitable to syrphids and butterflies, but bumblebees require a greater nectar supply and favour perennial forbs (Fussell and Corbet,1991a). Thus reseeding arable or resown field margins may not benefit bumblebees, despite providing nectar-producing flowers (Fussell and Corbet, 1991a). Semi-natural grasslands not only benefit nectar- and pollen-feeeders, but enable ground-nesting species such as bumblebees to establish colonies (Fussell and Corbet, 1991b). Ant-hill building species such as *Lasius flavus* also only survive in unploughed old pastures for the same reason (King, 1977).

Remote sensing to measure ecosystem services

It is becoming customary and deemed essential to employ new high-throughput technologies to enable the monitoring of large land areas for ecosystem service. Habitats are mapped for their potential for ecosystem services in accordance with definitions that arose out of the Millennium Ecosystem Assessment in 2005 (http://www.maweb.org/en/index.aspx). These are classified as either supporting (primary production, nutrient cycling and soil formation), provisioning (food, fuel, water), regulating (climate, flooding, disease) or cultural (aesthetic, spiritual, educational). Grassland habitats contribute value to all of these although, all too often, the cultural contribution is overlooked. In a study of the Spanish coast, the aesthetic contribution that was shared by a range of habitats was evaluated at >25% (Brenner *et al.*, 2010), as proof of its value.

The use of satellite images to identify habitats has been in use since the advent of the LANDSAT satellite series launched in 1982 (Xie *et al.*, 2008). As more satellites became available and resolution improved, extensive studies have been undertaken on large territories for land-use mapping, for instance, the EU Corine Land Cover initiative (http://www.eea.europa.eu/publications/COR0-landcover). In the UK, there is a bespoke land-cover/land-use mapping initiative using Landsat imagery by Centre for Ecology and Hydrology (CEH). This has identified several improved, semi-natural and natural grassland categories at the national scale. This is updated regularly, most recently in 2007 (http://www.ceh.ac.uk/landcovermap2007.html). However, for UK grasslands, the overall accuracy of the mapping can be very low, as little as 34% for some categories, due to the changing temporal nature of natural and semi-natural grasslands, and the impact of management practices such as grazing, silage removal and fertilizer application.

The advantage of using remote sensing to assess habitat quantity and quality is that it is repeatable over short time scales and covers large areas. China has been estimating the value of grassland ecosystem services countrywide using remote sensing (Jiang, 2007), and using non-market services such as measures for O_2 released, CO_2 fixed, control of soil erosion, water storage, nutrient recycling, reduction of pollution, and Net Primary Productivity. Results showed that habitats such as shrub-meadow complexes and upland grasslands provided the largest ecosystem service, while desert steppe and alpine desert provided the lowest.

Mapping at the landscape scale to define indicative habitat type and area can help with conceptual models of ecosystem services such as pollination. Classification of suitable satellite imagery can define grasslands of all types in terms of area and quality within a range of certainties. From these sources, patch and population-level attributes such as floral density, patch size and patch isolation can be determined, and will influence the interactions of the target plant communities with pollinators. A recent publication has promoted the use of a model called the Mobile-Agent-Based Ecosystem Service (MABES), where ecosystem services are reliant on a mobile organism to deliver a particular service (Kremen *et al.*, 2007). One worked example is pollination, but the model is being adapted for other tasks such as pest control and seed dispersal. Pollination is becoming an increasingly important ecosystem function due to the decline in bee populations worldwide. From a landscape perspective, a recent meta-analysis

found a significantly negative effect of habitat fragmentation on pollination of plants, and a strong correlation of this effect with reproductive success (Aguilar *et al.*, 2006).

Thus the fragmentation, and more specifically the isolation, of species-rich grasslands has wider negative effects on the ecosystem services they provide. On a landscape scale, agricultural intensification results in increased size of arable fields, decreased crop and weed diversity, and the loss and fragmentation of valuable natural to semi-natural perennial habitats such as agroforestry, grasslands and old fields. However, with more holistic grassland management at an integrated landscape-scale some more positive effects of agriculture on pollinator communities may occur. For example, in regions where the presence of low-intensity agriculture increases rather than decreases habitat heterogeneity within the foraging range of bees (e.g. <2 km), such as farmed landscapes that include relatively small field sizes, mixed crop types within or between fields, and patches of non-crop vegetation, such as hedgerows, fallow fields, meadows, and semi-natural wood or shrub-lands, it may be beneficial for biodiversity and ecosystem services (Bignal and McCracken, 1996; Sullivan *et al.*, 2011). Increased ecosystem services by grasslands needs to be properly managed and evaluated (Kremen *et al.*, 2007). For instance, what is required to best configure natural habitats within agricultural landscapes to promote population persistence of bees? How do the economic costs of these management practices compare to the benefits from enhanced pollination?

Breeding grasslands for crop production and carbon sequestration

A major aspect of ecosystem service by grassland is their role in C sequestration and in mitigating the rise in atmospheric CO_2 levels. Whether or not an ecosystem accumulates or loses carbon (both above and below ground) is a function of inputs and outputs. Sequestered carbon can be defined as the difference between gross primary productivity and ecosystem respiration, which in turn is the sum of plant respiration and heterotrophic respiration of non-photosynthetic organisms. This has been termed net ecosystem productivity (NEP) (Chapin *et al.*, 2006). The final rate of accumulation or loss of carbon in a particular ecosystem, in addition to NEP, will depend on external deposition of C (such as inputs of organic manures and dissolved C in rain water) and also losses through erosion, removal (harvesting) and non-biological oxidation through fire or UV radiation (Lovett *et al.*, 2006).

Grass crop breeding that accomplishes increased growth and turnover, both above and below ground, provides enhanced opportunity for C deposition. The original source of soil organic matter (SOM) is plant biomass, both above-ground, which decays in the litter layer and can be incorporated into the soil profile, and below-ground through turnover of the root system and as root exudates (Bardgett *et al.*, 2005). Various plant traits can affect the quantity and the quality, e.g. the C:N ratio of SOM, which in turn can affect its decomposition rates and the level of carbon retained as soil organic matter (De Deyn *et al.*, 2008). Traits that increase the rate of growth of above ground biomass, such as increased rates of photosynthesis, are often associated with a shorter lifespan and high nutrient demands, but this is not always the case. Often associated with high rates of photosynthesis and biomass production is a higher quality of the litter, which may lead to a faster turnover rate (Aerts and Chapin, 2000). For perennial crops such as grasses, their strategies for persistence and survival over years requires entry into cycles of growth in spring and early summer followed by reduced foliar and rooting growth in autumn. This may include periods of total growth cessation in preparation for winter survival. These annual growth cycles and associated growth conditions will inevitably impact on the rates of plant C deposition into soils. New strategies in Irish grassland agriculture that aim to make use of rising winter temperatures encourage whole-year growth and production. However, these have risk as severe crop damage may result from any onset of a harsh winter, and in any case will likely affect rates of accumulation of SOC. There is often trade-off between the amounts of biomass produced and its decomposition, characteristics which may affect soil organic

pools. Slower growing plants, in nutrient-poor environments, contribute to soil organic matter more on the basis of the recalcitrance of their organic matter, with high C:N ratios leading to slower rates of decomposition. In ecosystems with sufficient light and nutrients, and with high plant growth rate, the proportion of non-harvested biomass, and management regime such as cutting or grazing (where C is returned to the soil via dung) are major factors influencing levels of soil carbon. Grazing (defoliation) intensity and nutrient supply affect the balance of the major C fluxes and are best characterized in terms of the mean leaf area sustained over the growing season. 'Losses' of matter in respiration (approximately 40–50% of 'gross' photosynthesis) increase with the gross C uptake, of which 25% is associated with the energy and mass inefficiency of synthesis of new tissues, and the remaining 25% with the 'maintenance' of existing biomass (Thornley and Johnson, 1990). In perennial ryegrass, the longevity of leaves is approximately 30 days at 15ºC (Parsons et al., 2011).

Crops have been developed with different traits, and altered biochemistry from the wild type, through selective breeding programmes, hence leading to different litter qualities. Grasses with higher water soluble carbohydrate (WSC) contents, known as high sugar grasses (HSG), have been the focus of breeding programmes at IBERS (formerly IGER) to improve grassland productivity for meat and milk production (Humphreys and Theodorou, 2001). The impact of HSG and their potential for C sequestration is being investigated in collaboration with Rothamsted Research in the UK, but currently the 'jury is out'. The increased content of simple carbohydrates of HSG, as opposed to more recalcitrant compounds such as lignin, presents less of a challenge to decomposer organisms, a trait that also makes them more palatable for domesticated animals, and so will be decomposed more readily to CO_2 and make a lower contribution to soil carbon than more recalcitrant tissues. Among grass species it is the root litter that is more recalcitrant and offers a greater obstacle to decomposer organisms (Craine et al., 2005). The impact upon root litter quality of selective breeding for increased WSC content of shoot tissues is as yet unknown but whether or not there is a change in decomposability is likely to affect the positive or negative outcome this trait might have on SOM. Apart from root and shoot tissues, the other factor that can influence the sequestration potential of plants is root exudation, both the quantity and quality of the compounds that are exuded. It has been suggested that root exudation is governed by plant metabolic activity (Bardgett et al., 2005), with faster growing species producing more exudates. Again, the effect on root exudation of selection for a higher WSC content is little understood. Higher WSC in shoots may mean a higher concentration of WSC across all plant organs and in exudates. Alternatively, it could mean that the plant is more successful in partitioning WSC and concentrating them in shoot tissues and hence reducing concentration in roots and exudates.

Soils contain about twice as much C as the atmosphere and many soils are potentially able to sequester more than they do currently (Smith and Fang, 2010). Increasing steady-state soil C by 15% (e.g. from 0.05g/g to 0.058 g/g), it is claimed (Kell, 2011) would lower atmospheric CO_2 by 30%, leading to a large environmental benefit. Deep soil carbon is an important contributor to overall soil carbon stocks with grasses and trees acting as major sources (Chabbi et al., 2009; Harper and Tibbett, 2013). Gregory et al. (2011) estimated that 980 Mt of organic C is stored below 30cm depth in soils in England and Wales, approximately 50% of the total. Evidence from tropical savannah suggests that the planting of exotic deeper rooting plant varieties leads to significant increases in soil carbon (Fisher et al., 1994). It is helpful that a grass trait that has received recent attention by crop geneticists for a range of reasons is for deeper rooting, with focus particularly on Festulolium (Kell, 2011). These are hybrids or hybrid derivatives between any Festuca (fescue) and Lolium (ryegrass) species, designed for their combined complementary characters (Ghesquière et al., 2010). The potential of certain Festulolium hybrid combinations for root growth and turnover to enhance SOC in sub-soils is currently being investigated and it may extend the range and diversity of soil biota. The deep-

rooting trait was originally selected as an aid to an improved drought resistance (Durand *et al.*, 2007; Alm *et al.*, 2011), but also provides other ecosystem services, such as improving soil water retention and hence reduced runoff (Gregory *et al.*, 2010; Macleod *et al.,* 2013). The cultivation of *Festulolium* with large and deep-rooting systems as an aid to increase the input of atmospheric CO_2 into agricultural soils is being assessed currently (J. Dungait, pers. comm.). However, there is conflicting opinion on whether the stores of SOC in subsoil will be increased or decreased by the introduction of new C. In addition, in order for deep-rooting plants to have any significant effect, the soil must be deeper than the root depth of current varieties, and furthermore root growth must not be impeded due to compaction, nutrient limitation or waterlogging. On thin soils or where roots cannot penetrate, deeper rooting plants are unlikely to provide additional sequestration benefits. Also, some studies have pointed to the effect of 'priming' whereby fresh labile organic matter stimulates the decomposition of older soil organic matter through the soil profile (Kuzyakov, 2010) and more specifically at depth (Fontaine *et al.*, 2007). What happens to soil C at depth during disturbances such as drying or ploughing, land use change *etc.* is still little understood (Gregory *et al.*, 2011).

From an agricultural perspective, it is essential that a balance between above- and belowground growth is achieved so that forage yields are not significantly compromised. Recent studies at IBERS have demonstrated that *Festulolium* hybrids involving combinations of *L. multiflorum* or *L. perenne* with either *F. arundinacea var glaucescens* or *F. mairei* have excellent agronomic performance, including high yields of forage together with high WSC, and DOMD. From the perspective of their C sequestration potential, they also produce deep root systems that match their high above-ground growth. The *L. perenne* x *F. pratensis* amphiploid variety Prior has deep rooting and significant root turn-over at depth (MacLeod *et al.,* 2013) leading to potential ecosystem benefits in terms of improved soil porosity. Dungait (pers. comm.) has provided preliminary data supporting greater root depth and C deposition by *Festulolium* cv. Prior in soils, compared with that of perennial ryegrass.

Impacts of climate on C sequestration

There have been extensive reviews (e.g. Soussana and Lüscher, 2007) of the impact of multiple components of climate change, singly or in combination, on the fluxes of C and N. As the major resource for photosynthesis, the expectation borne out by field experimentation is that elevated CO_2 will increase all plant-derived C fluxes into the system. An increased uptake and availability of C due to elevated CO_2 has been shown to increase C:N ratios in tissues within pasture species (Poorter *et al.*, 1997) and to increase the exudation from roots of labile C (Allard *et al.*, 2006). However, over an 11-year period of CO_2 enrichment of grazed pastures, Newton *et al.* (2010) found a gradual transfer of N from plant to soil pools which would undoubtedly be a constraint on plant growth responses to elevated CO_2. Overall, models predict higher total soil C sequestration in grasslands at elevated CO_2 (Pepper *et al.*, 2005).

A recent study on Oklahoma prairie grasslands has shown that complex interactions occur in microbial diversity in soils in reaction to warming and drought. Warming without drought had the effects of increasing the abundance of microbes, but had the effect of reducing their diversity. Warming with drought resulted in large reductions of microbial populations but diversity was not affected. Some microbial communities are very resilient and recover quickly after the stress is removed, but others are not, becoming dormant and requiring significant time to reinstate after the stress had been removed (Sheik *et al.*, 2011).

Soil microbial composition is affected directly by the accompanying plant species in grasslands. Grigulis, *et al.* (2013) showed that high biomass production by plants was accompanied by bacterial dominance in the soil, fast microbial processes and a competitive strategy. By contrast, plant traits exhibiting low biomass production were associated with an increase in fungal communities, and slow microbial turnover. The low biomass communities

were therefore conservative by nature, providing ecosystem services such as high nutrient retention but lower C sequestration, while the more exploitative plant communities provided greater Net Primary Productivity and more C sequestration.

Redesigning grassland crops for improved adaptations to climate change and for ecosystem service

Comparative studies of genetic and phenotypic diversity found in natural grasslands adapted to contrasting climates provides us with insights into the key mechanisms involved in resistance to specific abiotic stress extremes, and reveals components of their genetic control. By selection and transfer from adapted ecotypes of trait-specific alleles, centuries of evolved adaptations may frequently be utilized over a few generations to reproduce a comparable phenotype in a commercial cultivar. For a perennial grassland species, not only is cultivar performance and stability over years important, but also its ability to coexist within complementary species' mixtures and its competitive capabilities against invasive species. An extensive literature has explored the basis of grass-clover competition, with both above and below-ground factors found to be important. Shading of clover by grass can occur, depending on clover leaf size, management and, crucially, soil nitrogen (N) status. There is thus a strong interaction between interspecific plant competition and soil, particularly for the clover-*Rhizobium* symbiosis. Hydraulic lift is another positive species interaction that could be incorporated in species mixtures. The phenomenon, reported initially amongst tree and shrub species, describes how shallow-rooting species can benefit from improved acquisition of water and nutrients because of the release of water and nutrients into the topsoil from deep-rooting neighbours (e.g., Snyder *et al.*, 2008).

Advances in plant breeding strategy at IBERS over recent years have provided productive grass and clover varieties that also safeguard the environment. In the forefront are the high sugar ryegrasses (HSG) described earlier, and also legumes designed to improve ruminant nutrition by more efficient protein conversion and thereby to reduce greenhouse gas emissions by livestock. Two possible strategies of increasing efficiency of conversion of forage N to microbial N have been used: (i) increasing the amount of readily available energy accessible during the early part of the fermentation; and (ii) providing a level of protection to the forage proteins, and thereby reducing the rate at which their breakdown products are made available to the colonizing microbial population. The HSG are examples of the former, where increased WSC has been shown to have a positive impact on meat yields (Lee *et al.*, 2001) and milk production (Miller *et al.*, 2001). A significant contributor to greenhouse gas emissions by livestock is the plant-mediated proteolysis that occurs as a consequence of the assorted stresses encountered by living cells of ingested forage while in the rumen (Kingston-Smith *et al.,* 2010). The second strategy to improve ruminant nutrition has focused on this, with a role for *Festulolium* in protein protection having been identified (O'Donovan *et al.*, 2013). Amphiploid hybrids involving either *L. multiflorum* or *L. perenne* together with *Festuca arundinacea var glaucescens* have highly significantly enhanced protein stability compared with their *Lolium* parents when subjected to rumen-simulated conditions. It is hypothesized that protective measures that have evolved in the fescue to combat heat stress in its natural Mediterranean habitat, also come into play in the rumen to provide protein protection and to provide greater time for breakdown by rumen-based microbial populations.

Plant breeding initiatives to improve protein stability under rumen conditions and to reduce greenhouse gas emissions by livestock have also involved legumes, such as polyphenol oxidase (PPO) expression by red clover (*Trifolium pratense*) (Webb *et al.*, 2013). Condensed tannins also help to stabilize protein as it passes through the rumen, reducing loss of protein and preventing bloat. Although absent from the leaves of white and red clover, they are present in *Lotus corniculatus* and *Lotus uliginosus* (Marshall *et al.*, 2008). Extensive variation in

condensed tannin content of the leaves of *Lotus* has been found. The development of varieties with appropriate levels of condensed tannins, to reduce protein loss without reducing intake or the agronomic yield and persistence is a research objective.

Developing grasses with increased resilience to climate change

Reference has already been made of the potential of *Festulolium* for ecosystem service. There are several examples of natural *Festulolium* hybrids. For example, both Italian ryegrass and perennial ryegrass hybrids with meadow fescue occur naturally in the UK as *Festulolium braunii* and *Festulolium loliaceum*, respectively, and have evolved with certain adaptive capabilities superior to their parent species. *Festulolium loliaceum* for example, is found commonly in mature meadows in flood-prone highly water-logged soils (Humphreys and Harper, 2008). IBERS achieved a notable first in 2012 in gaining inclusion onto the UK National Recommended Varieties List of the *Festulolium* variety AberNiche, which is a modified *F. braunii* comprising circa 10% meadow fescue genome (based on genetic and cytological screens fescue-specific using DarT markers and genome in-situ hybridization (GISH) (Kopecky and Harper, unpublished). The variety was developed for its high forage production and improved winter hardiness, presumably derived from its cold-tolerant fescue progenitor, but has also demonstrated excellent resilience to heat and drought.

The entry of variety AberNiche onto the UK National Recommended Varieties List heralds a new dawn for *Festulolium* breeding in the UK. Genes for drought resistance have been transferred successfully from both *F. arundinacea var glaucescens* and from *F. arundinacea* onto chromosome 3 of both *L. multiflorum* and *L. perenne* and have improved the water use efficiency of both ryegrasses by >80% (Humphreys *et al.*, 2013). Breeders' lines with these fescue gene complements are currently in trial for variety assessment.

Impact of flooding and drought

The use of *Festulolium* for flood mitigation has been recently proposed (Macleod *et al.*, 2013). In the UK, the cost of flooding is significant; it has been estimated that the devastating floods of summer 2007 cost the UK economy £3.2b (Environment Agency). Excessive run-off erodes top-soils and soil organic matter, and depletes valuable nutrients, with negative impacts on water quality. Eutrophication of surface and ground-waters in England and Wales is estimated to cost £75-114m a^{-1} due to loss of amenity value, reduced biodiversity and increased costs of water treatment. The cost of damage to agricultural soil in England and Wales has been estimated as £264m a^{-1}, and that of treating water contaminated with agricultural pollutants as £203m a^{-1} (UK Parliamentary Office of Science & Technology, 2006). The winter of 2013-14 brought record rainfall to the UK leading to extreme flooding events across largely grassland-dominated areas. Insurance losses for England and Wales are expected to amount to £1.2b. Over the UK as a whole £600m of crops were lost due to flooding in 2012. To help mitigate the worst effects of flooding, *Festulolium* hybrid combinations are proving effective. Macleod *et al.* (2013) demonstrated how *L. perenne* x *F. pratensi*s variety Prior reduced rainfall surface run-off by 51% compared to an IBERS-bred elite perennial ryegrass variety. It is hypothesized that the root turn-over at depth of the *Festulolium* led to improved soil structure and porosity and to improved soil water retention and reduced overland run-off. In a new five-year project (2014-2018) called SUREROOT, funded jointly by BBSRC and industry, IBERS and Rothamsted Research are assessing the potential of *Festulolium* and clover varieties both independently and in combination for flood mitigation. The trial will include field assessments on farms at different UK locations, on contrasting soils, and with alternative livestock management practice. The project also employs use of IBERS new National Plant Phenomics Centre and the North Wyke Farm Platform to assess how modified root systems on individual plant genotypes may if reproduced at the field scale affect soil structure and water, nutrient,

and C run-off at the field scale. Similar to *Festulolium* cv Prior, white clover improves soil structure and drainage through improved soil aggregation, which increases soil porosity and water infiltration (Mytton *et al.*, 1993; Holtham *et al.*, 2007). Values of macroporosity for soil cores under perennial ryegrass and under white clover were reported as 24% and 45%, respectively, indicating a significant improvement in water drainage through use of white clover (Holtham *et al.*, 2007).

Festulolium for bioremediation on mine-spoil

Whilst primarily for economic reasons, mining underground for coal in the UK is largely no longer practised, it has been replaced by open-cast mining in areas such as South Wales, a practice viewed as a controversial due to its unsightly look, the large land areas employed, and the potential damage to both landscape and environment. It is considered a priority in such locations that areas used for open-cast mining are restored to their natural grassland ecology as quickly as possible as soon as coal extraction has ceased. However, combinations of a challenging climate and growth inhibiting mine-spoils have restricted progress to land reclamation. However, new *Festulolium* combinations: *L. perenne* x *F. pratensis, L. perenne* x *F. arundinacea var glaucescens,* and *L. perenne* x *F. mairei* amphiploid hybrids are being assessed by IBERS in trials on over-burden mounds on open-cast mine workings in South Wales aimed at their bioremediation and their restoration to grasslands. The combination of stress tolerance, rapid establishment and growth, nutrient and water-use efficiency, and large strong root systems present in the *Festulolium* hybrids provide them with an advantage over native grasses. Their rapid root turn-over should provide new sources of C to the mine spoil and provide the foundation for indigenous UK grasses to colonize and eventually return the land finally to its natural condition.

Conclusion

The development of new grassland agro-ecosystems must take account not only of traditional values of production, disease resistance, persistence and forage quality but also their impact on their surrounding environment. National variety assessment needs to be amended to account for the potential benefits to accrue through the use of novel varieties that offer enhanced resilience against climate change and/or provisions for other ecosystem service. A cohesive strategy is required involving relevant stakeholders to inform, encourage, and reward farmers to grow grassland varieties that provide a specific ecosystem service such as flood mitigation; to provide the necessary guidance as to where to sow, and how to manage sufficiently to produce the optimal benefits.

Anthropomorphic and natural grasslands can potentially be mutually advantageous. Whilst historically agricultural developments have diminished areas of natural grassland and have led to the loss of their traditional environmental provisions, a reverse result may also be achieved. Novel grassland varieties have potential to engineer conditions that allow the restoration of natural grassland ecosystems, e.g. in land reclamation from industrially contaminated 'brown-field sites'. They may also extend the depth of sub-soils and extend the range of soil biota by rooting deeper than indigenous species. Indeed they may in species mixtures increase the resilience of grassland ecosystems to climate change in situations where the indigenous species lack the necessary adaptations to the stresses likely to be encountered. The genetic resources of many semi-natural grassland species can be harnessed to bring both the productive and 'unproductive' grasslands more into line. Semi-natural grasslands should not be showcased and 'fossilised in time and place' but must be preserved as essential resources for genetic material for future crop improvement and for ameliorating the damage done by traditional productive systems. Restoration at the landscape scale is required to redress the balance and

provide more connectivity, more ecosystem services, and more multifunctional dynamism in grassland management.

Whilst new grassland varieties are required to reduce our 'agricultural footprint', to safeguard the future of fragile rural communities and to sustain our livestock industry, it is essential that further safeguards are put in place to assist the perpetuity of our existing natural and semi-natural grasslands. In the current drive to increase productivity, natural grasslands are in danger of being further eroded and fragmented, greatly reducing their biodiversity and their ecosystem service value. They it must be remembered provide a much more diverse range of essential ecosystem services than those available within agricultural crop based systems. Indeed, restoration of partially degraded grasslands should also form part of national objectives. Grasslands, both natural and cultivated, dominate our landscape and both have a vital role in safeguarding the environment on which we all depend.

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Theme 2 submitted papers

Single species and mixed grazing regimes to restore *Nardus stricta* moorland

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Abstract

Upland heathlands provide a range of ecosystem services but have been degraded by high stocking densities, causing replacement of dwarf shrubs by grasses such as *Nardus stricta*. A long-term paddock-scale experiment is being conducted to assess the effects of sheep, cattle and mixed grazing regimes on vegetation and livestock performance on former dwarf shrub heathland dominated by *N. stricta*. Treatments of 1.0 and 1.5 ewes ha^{-1}, 0.5 cattle ha^{-1} in summer, and mixed grazing comprising 1.0 ewes ha^{-1} plus 0.5 cattle ha^{-1} in summer, have been applied over ten years, including a two-year period with no grazing, representing a 'pulsed' grazing system. Across all treatments, *N. stricta* frequency declined and this continued during the two years with no grazing, but the decline was not mirrored by any increase in dwarf shrubs. In the first year when grazing was re-introduced, cattle and sheep liveweight gains were satisfactory for commercial production and at least comparable to gains during previous years of the experiment. Pulsed grazing regimes with sheep or cattle at low to moderate stocking densities could be viable, but with beneficial effects on vegetation only emerging over several years.

Keywords: *Nardus stricta*, dwarf shrubs, upland heathland, sheep, cattle, mixed grazing

Introduction

Upland heathlands in the UK are an internationally important habitat, providing a range of ecosystem services including biodiversity and food from extensive livestock production, as well as flood regulation, carbon storage and recreation. Past high stocking densities, particularly of sheep in the second half of the 20th century, caused degradation of upland heathland in many parts of the UK. This resulted in a loss of dwarf shrub species such as *Calluna vulgaris* and *Vaccinium myrtillus* and their replacement by grasses such as *Nardus stricta*. Since the late 1980s, agri-environment schemes in the UK have encouraged farmers to reduce sheep numbers in an attempt to reverse this process. However, cattle consume *N. stricta* more readily than do sheep, and could play a positive role in altering competitive interactions between plant species. Rotational or 'pulsed' grazing systems, in which an area is left ungrazed for up to 3-4 years between periods of grazing, could also help to restore upland heathland (Gardner *et al.*, 2009). A long term paddock-scale experiment has been set up to assess the effects of sheep, cattle and mixed grazing regimes on biodiversity and livestock performance when applied to an area of *N. stricta* acid grassland. Here we examine the effects on vegetation and livestock performance following a two-year period without grazing, as part of a long term pulsed-grazing system.

Materials and methods

The study site was 71 ha of *N. stricta* grassland at an elevation of 305–625 m above sea level, with annual rainfall c. 2000 mm, in mid-Wales, UK (52° 22' N 3° 46' W). Soils were Stagnopodzols with peaty top soils or shallow soils with bedrock at 30 cm. The area was formerly dwarf shrub heath but degraded by a long period of sheep grazing at c. 2.2–2.7 ewes per ha up to the mid-1990s. The vegetation resembled variants of the U5 *N. stricta – Galium saxatile* grassland (Rodwell, 1992). The site was divided into twelve paddocks and four grazing

treatments applied from spring 2003 to autumn 2010, allocated randomly to paddocks and replicated over three blocks. All livestock were then removed for two years before re-introducing the grazing treatments in 2013. Treatments were low sheep (LS; 1.0 Welsh Mountain ewes ha^{-1} for ten months per year + lamb from May to August), high sheep (HS; 1.5 Welsh Mountain ewes ha^{-1} for ten months per year + lamb from May to August), cattle only (CO; 0.5 Welsh Black 2-year-old heifers ha^{-1} for two months in July and August) and mixed sheep plus cattle grazing (SC; low sheep + cattle only regimes).

A grid of 125 points at 75 m spacing was superimposed on the study site and vegetation recorded at each point from a fixed 1 m × 1 m quadrat subdivided into 100 cells of 10 cm × 10 cm. Local shoot frequency (presence in each 10 cm cell) and a grazing index (proportion of occupied cells in which grazed shoots were present) of four key species (*N. stricta, V. myrtillus, C. vulgaris, Molinia caerulea*) had been recorded annually from 2003 to 2006, and this was repeated in 2010 and 2012. The maximum sward height within each quadrat was recorded using a sward stick. Vegetation records were made in September–October. Liveweights were recorded for all heifers when turned onto and removed from the paddocks, and for ewes from the onset of grazing in June 2012 to weaning in August. Lambs were weighed before turning on to the paddocks in June and again at shearing in July and at weaning in August. All data were analysed using repeated-measures Analysis of Variance in Statistica v11[©] (Statsoft Inc., Tulsa, Oklahoma).

Results and discussion

Mean vegetation height increased across all treatments during the period without grazing, but only by *c.* 2 cm, just outside the limits of statistical significance ($F_{1,6} = 5.2$, $P = 0.06$). Height variability (coefficient of variation) appeared to decline overall ($F_{1,6} = 5.2$, $P = 0.06$). Background grazing levels from wild herbivores in 2012 were negligible, in contrast to the previous years with livestock when, for example, grazing indices were $21-23\%$ on *V. myrtillus* in treatments with sheep, and $54-63\%$ on *M. caerulea* in treatments with cattle. *N. stricta* frequency appeared to decline during the period with no grazing, from 37.6% in 2010 to 30.8% in 2012 ($F_{1,6} = 5.5$, $P = 0.06$). This occurred across all treatments (year x treatment interaction not significant) and continued a decreasing trend recorded previously, from 51.4% in 2003 to 36.8% in 2005 (Critchley *et al.*, 2013) (Figure 1).
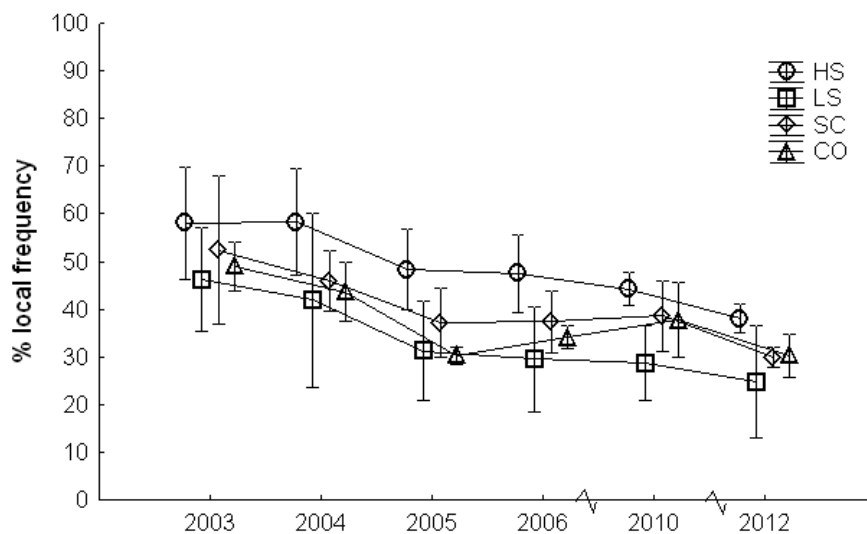
Figure 1. Local frequency (means and standard errors) of *N. stricta* in the four grazing regimes. HS = high sheep; LS = low sheep; SC = sheep + cattle; CO = cattle only

This trend could be a long-term response to the reduced numbers of livestock on the site during the experiment, which is likely to increase competition on *N. stricta* from more palatable grasses (Gardner *et al.*, 2009). *M. caerulea* was only present at low frequencies but showed a significant increase from 4.4% to 6.7% across all treatments during the no-grazing period ($F_{1,6}$ = 11.5, $P < 0.05$). However, no changes or other effects were detected for *C. vulgaris* or *V. myrtillus* frequencies.

In the first year when grazing was re-introduced (2013), cattle liveweights increased significantly ($F_{1,20} = 120.3$, $P < 0.001$) during the period on the paddocks. Weight gains were slightly greater in the CO treatment (0.9 kg day^{-1} +/- 0.40 st.dev.) than SC (0.6 kg day^{-1} +/- 0.20 st.dev.) ($F_{1,20} = 5.4$, $P < 0.05$), and were considered adequate for commercial production. Cattle liveweight gains in 2013 compared favourably with those during the period 2003 to 2010, when overall mean daily liveweight gains were 0.6 kg day^{-1} (CO) and

0.5 kg day^{-1} (SC). This might be attributable to an increase in availability of forage biomass following the two years without grazing, although younger heifers were used in 2013, with lower mean initial liveweights (334 kg) compared to the period 2003 to 2010 (401 − 536 kg), and more potential for growth. Ewe liveweights increased significantly ($F_{1,59} = 6.1$, $P < 0.05$) from the onset of grazing in June 2013 to weaning in August (15 g day^{-1} +/- 50.6 st.dev.) but there were no significant differences between treatments. Lamb liveweight increases from the time on the paddocks up until shearing in July were lower in the HS treatment (132 g day^{-1} +/- 36.7 st.dev.) than LS (163 g day^{-1} +/- 49.3 st.dev.) and SC (177 g day^{-1} +/- 57.5 st.dev.) ($F_{2,58}$ = 5.6, $P < 0.01$). However, there were no significant treatment effects on liveweight gains between shearing in July and weaning in August, nor on actual weaning weights (25 kg +/- 4.1 st.dev.), which were comparable to those from 2004 to 2010 (*c.* 24 − 32 kg overall).

Conclusion

Initial results suggest that pulsed grazing regimes with sheep or cattle at low to moderate stocking densities could have some small beneficial effects on vegetation without compromising livestock performance but major changes are likely to take much longer.

Acknowledgements

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Integrating biodiversity conservation with grassland farming: extensive cattle grazing and farmland birds

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Abstract

Extensively grazed pastures are a valuable habitat for invertebrate-feeding birds and thus form a key component of agri-environment schemes that aim to restore declining farmland bird populations. Extensive grazing measures are potentially costly to implement as agricultural outputs can be impaired. The conflicting demands for food production (or cost to the agri-environment scheme) and for conservation measures to be effective can be reconciled if the trade-offs between their different requirements are understood. This paper describes how an effective, low-cost extensive grazing measure was developed, by experimentally manipulating cattle grazing pressure to promote selective grazing and increase structural heterogeneity. The solution proved to be simple and flexible. Managing stocking rates to maintain an average sward height in the range 9-12 cm provided valuable foraging habitats for threatened farmland birds, while keeping agricultural costs well within available conservation budgets. The measure has been adopted by the English agri-environment scheme.

Keywords: agri-environment measures, birds, extensive grazing

Introduction

A high proportion of the UK farmland bird species that are in severe long-term decline require invertebrate prey to rear their young. Extensively grazed pastures are an important source of suitable prey and play an important role in maintaining reproductive output in species such as the yellowhammer (*Emberiza citrinella*) and cirl bunting (*E. cirlus*) (Buckingham, 2006). Extensively grazed pastures form a key component of agri-environment schemes aiming to maintain or restore farmland bird populations, but they are potentially costly because suitable management curtails livestock production. This paper describes the research behind the development of an effective but affordable extensive grazing measure. The optimization process was guided by learning how structural heterogeneity in the sward can simultaneously meet the conflicting requirements of foraging birds, their invertebrate prey and livestock. This structural heterogeneity was created by reducing grazing pressure to encourage cattle to graze selectively (Hofmann and Tallowin, 2004).

Materials and methods

Extensive grazing treatments were compared in a series of split-field experiments between 2006 and 2012. In each experiment, all treatments were replicated in each study field, in 1-ha paddocks separated by fences. The paddocks were grazed by cattle and stocking rates were manipulated to maintain target sward heights (TSH). Sward structure, biodiversity metrics (plant and invertebrate communities; bird usage) and livestock live-weight yields were measured at the paddock level to quantify treatment effects in each year of the experiment. Variation within paddocks was measured to investigate the role of structural heterogeneity. The first experiment (2006-2009) contrasted a commercially grazed control (TSH 6-8 cm, April-October) with early closure (cattle removed in mid July) at two levels of grazing pressure: moderate (TSH 7-9 cm) and lenient (TSH 12-15 cm) (Peach, 2010). The experiment

was conducted on 13 unfertilized, semi-improved, permanent pastures. A subsidiary experiment tested these treatments for two years on 8 agriculturally improved permanent pastures with a recent history of fertilizer applications $\geq$50 kg N ha^{-1} year^{-1}. The second experiment (2010-2012) compared the same commercially grazed control with two treatments which were kept at the same intermediate TSH (9-12 cm), by grazing continuously or intermittently (as in a rotational paddock grazing system) (Buckingham, 2013). All fields were situated in Devon, SW England and received no fertilizers during the experiments.

Results and discussion

The first experiment showed that lenient grazing combined with early closure led immediately to increased invertebrate densities in the first year, with further cumulative increases over time (Peach, 2010; Eschen *et al.*, 2012). During the breeding season, invertebrate-feeding birds significantly preferred the extensively managed treatments as they held the most prey taxa. These patterns were observed on semi-improved and improved pastures. However, the lenient early-closure treatment caused substantial live weight yield losses (32% in year 2, rising to 62% in year 4) and key conservation priority bird species (notably the *Emberiza* buntings) did not respond strongly to the treatments. Partitioning the treatment responses into their constituent management components showed that early closure caused the biggest yield reductions through the loss of late-season grazing. Both early closure and lenient grazing also increased less-productive grasses, particularly *Holcus* and *Agrostis*, at the expense of ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). Lenient grazing resulted in the largest increases in invertebrate densities. Contrary to expectation, structural heterogeneity fell over time on both extensively managed treatments and fell below levels required by foraging buntings, explaining why usage levels were so far below those observed in other grassland studies (Stephens *et al.*, 2002; Buckingham, 2006). Early closure failed to provide an anticipated increase in the availability of large-bodied invertebrate prey taxa in the early part of the bunting breeding season. The extensive grazing treatments mainly benefited birds in the second half of the breeding season.

Based on these results, early closure was abandoned because of its high agronomic costs and smaller biodiversity benefits, compared to lenient grazing. Grazing throughout the growing season at a lower TSH was predicted to reduce yield losses and sward deterioration and to increase structural heterogeneity in the sward, thereby increasing its utility to foraging buntings. An intermediate TSH (9-12 cm) was adopted in an attempt to retain elevated invertebrate prey densities and high usage by foraging and nesting skylarks, all of which were predicted to decline in shorter swards. The resulting prediction was tested in the second field-scale experiment.

The second experiment confirmed that intermediate TSH grazing significantly increased invertebrate densities and the utility of pastures to both buntings and skylarks (Buckingham, 2013). The agricultural costs of intermediate TSH grazing were low in the first two years (£37-71 ha^{-1} year^{-1}). However, the third year (2012) was adversely affected by prolonged high rainfall, leading to a sharp increase in yield losses (£139 ha^{-1} year^{-1}) and reduced biodiversity benefits. It is not clear whether agricultural costs would remain low for longer, in the absence of extreme weather, but these figures places bounds on the likely costs in adverse conditions. In practice, there were numerous interruptions to the continuity of grazing, particularly during dry conditions in the first two years, so the continuous and intermittent treatments differed mainly in the duration and frequency of grazing interruptions. Both continuous and intermittent grazing treatments resulted in similar biodiversity benefits on intermediate TSH paddocks, but invertebrate densities and bird usage tended to be lower on the intermittent grazing treatment. There was no evidence that the intermittent treatment enhanced sward heterogeneity.

Conclusion

Having been proven effective and affordable, intermediate TSH grazing will be funded through the new English agri-environment scheme (NELMS: the New Environmental Land Management Scheme, beginning in 2016). The 9-12 cm TSH allows farmers to adapt their stocking levels to local conditions, including seasonality of grass growth and weather. Additional management flexibility is allowed where evidence from the experiments indicates that it will not compromise the biodiversity objectives. The principal uncertainty is how long the intermediate TSH swards can be maintained without swards or yields deteriorating, particularly if extreme weather occurs. The new measure allows farmers to remove a field from this option after two years, if necessary. The measure is then implemented on a replacement field, so that an agreed area of measure is present on the farm throughout the NELMS agreement. Where possible, farmers are encouraged to continue managing fields with intermediate TSHs for longer, as the biodiversity benefits are expected to be cumulative. The measure also allows continuous or intermittent (paddock) grazing and small applications of farmyard manure, as the evidence suggests that these changes do not impair the biodiversity benefits. The area of this measure that is required to benefit a bird species at the population level is unknown. However, a single field managed correctly will benefit bunting territories within 200-400 m (Stevens *et al*., 2002), so only a small area within a farm needs to be committed to this measure, for it to have an effect.

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Spatial soil variation on the North Wyke Farm Platform

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Abstract

Rothamsted Research at North Wyke in the south-west of England hosts the Farm Platform, a unique research facility for sustainable grassland systems. Fifteen catchments are distributed between three farmlets, ear-marked for different future land uses from April 2013. In order to check that the catchments had been allocated equally between the three farmlets with regards to quality, the soil was surveyed for each catchment in June 2012. Soil parameters of bulk density, pH, total nitrogen, total carbon and organic matter were surveyed on a square 50 m grid system and catchments differed from poorer soils to better soils with respect to the parameters surveyed. Catchments with sampled means at either ends of the range displayed consistency, producing a lower coefficient of variation than catchments with sampled means in the middle of the 15-field range. The means and coefficient of variation showed that the allocation of catchments to each farmlet gave an even share of catchments to each future land use.

Keywords: farm platform, soil nutrients, spatial variation, grassland

Introduction

Advanced technology in the field of auto-measurement has a major role to play in agri-research (Griffith *et al*., 2013), but a key requirement is to start with a knowledge of baseline conditions (Orr *et al*., 2011) before measuring the effects of treatments. Rothamsted Research at North Wyke hosts the Farm Platform which contains 15 catchments consisting of one or more fields on permanent pasture which are hydrologically isolated and auto-sampled for drainage runoff and soil moisture, and monitored for agronomic productivity of beef and sheep systems. The dominant soil type at North Wyke is Hallsworth and Halstow series clay (Harrod and Hogan, 2008) with an impermeable layer at 30 cm depth, and runoff is collected at the perimeters leading to a flume laboratory where water flow and chemical/physical properties are measured. Catchments were allocated to three farmlets (Figure 1) from April 2011 for two years, after which they began to change to different land managements.

Materials and methods

Unstratified regular square grid sampling of soil was conducted in June 2012 in all the catchments on a fixed grid of 50 m with known GPS coordinates and 243 soil samples were collected and analysed. Bulk density was determined in samples which were collected by pressing a cylindrical core with 5.5 cm diameter and 10 cm depth into the soil. Samples were sieved to separate vegetation and stones larger than 2 mm and the volume of these was then measured by displacement of water. The remaining soil was oven dried at 105°C for 24 h and bulk density was calculated. Total nitrogen, total carbon and organic matter and pH were measured at each sampling point in 10 soil cores which were 2 cm diameter and 10 cm depth. These were mixed and sieved though 2 mm mesh, ground and oven dried for 24 h. Analysis was conducted using an elemental analyser (NA2000, Carlo Erba Instruments, Milan, Italy) and soil organic matter was determined by loss on ignition. Soil pH was determined by adding 25 ml of deionised water to 10 ml of soil and measuring the solution pH.
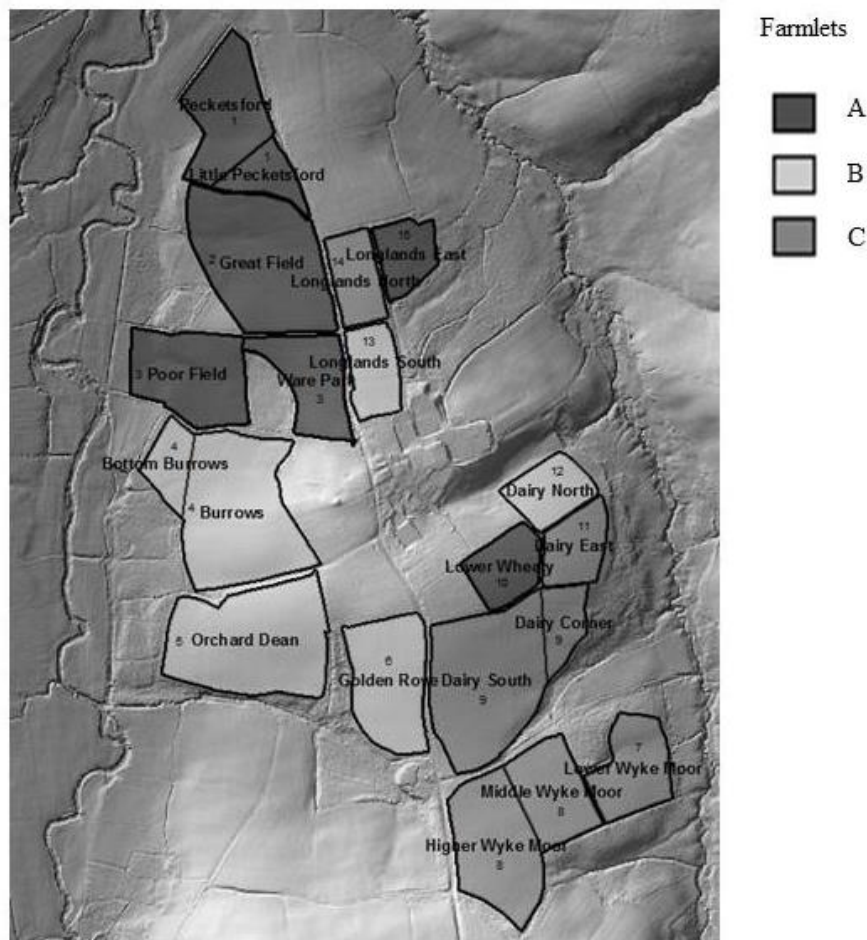
Figure 1. The North Wyke Farm Platform showing grey-scale farmlets each with 5 numbered catchments containing one or more fields.

Results and discussion

The mean sampled bulk density (Table 1) varied from 0.8 (coefficient of variation 0.10) to 0.98 (c.v. 0.07) with a catchment range of c.v. from 0.05 to 0.13. The farmlet means ranged from 0.86 to 0.92. Soil pH varied from 5.28 (c.v. 0.02) to 6.15 (c.v. 0.02) with a catchment range of c.v. from 0.01 to 0.08. The farmlet means ranged from 5.49 to 5.68. Total nitrogen varied from 4.27 (c.v. 0.07) to 6.79 (c.v. 0.04) with a catchment range of c.v. from 0.06 to 0.20. The farmlet means ranged from 5.28 to 5.59. Total carbon varied from 31.24 (c.v. 0.04) to 58.80 (c.v. 0.07) with a catchment range of c.v. from 0.04 to 0.23. The farmlet means ranged from 46.04 to 46.65. Organic matter varied from 6.78 (c.v. 0.11) to 12.46 (c.v. 0.05) with a catchment range of c.v. from 0.04 to 0.23.

The farmlet means ranged from 10.04 to 10.48. In all cases the catchments showing highest and lowest parameters displayed lower variations, hence the highest and lowest means were the most stable. The three Longlands fields included between them the highest bulk density and pH, with the lowest nitrogen carbon and organic matter. Meanwhile, fields used previously for dairying contained higher organic matter, total carbon and total nitrogen content; e.g. Lower Wheaty and Dairy North. Both the Longlands fields and the former dairying fields were shared amongst each farmlet, which was reflected in the farmlet nutrient results being evenly distributed.

Table 1. Soil parameters sampled in 2012 and mean values per catchment[*] and per farmlet (with coefficient of variation).

Catchments	BD (g/cm³)	pH	Total N (g/kg)	Total C (g/kg)	SOM (mg/litre)
FARMLET A					
Pecketsford & Little Pecketsford	0.92 (0.09)	5.47 (0.03)	6.05 (0.09)	49.84 (0.10)	11.20 (0.10)
Great Field	0.97 (0.12)	5.88 (0.05)	4.28 (0.13)	34.33 (0.15)	7.73 (0.11)
Poor Field & Ware Park	0.85 (0.07)	5.62 (0.08)	5.41 (0.15)	44.30 (0.17)	10.83 (0.16)
Lower Wheaty	0.91 (0.04)	5.28 (0.02)	6.79 (0.04)	57.54 (0.05)	11.82 (0.06)
Longlands East	0.96 (0.05)	6.15 (0.02)	4.37 (0.11)	35.98 (0.16)	9.35 (0.10)
FARMLET B					
Burrows & Bottom Burrows	0.91 (0.09)	5.67 (0.06)	5.35 (0.11)	44.15 (0.12)	8.93 (0.23)
Orchard Dean	0.92 (0.10)	5.79 (0.04)	5.82 (0.12)	51.74 (0.15)	12.08 (0.13)
Golden Rove	0.94 (0.07)	5.59 (0.06)	5.84 (0.08)	50.15 (0.07)	10.97 (0.04)
Dairy North	0.92 (0.03)	5.79 (0.02)	6.55 (0.05)	58.80 (0.07)	12.46 (0.05)
Longlands South	0.98 (0.07)	5.48 (0.01)	4.56 (0.06)	38.08 (0.08)	8.76 (0.07)
FARMLET C					
Higher & Middle Wyke Moor	0.84 (0.08)	5.38 (0.06)	4.53 (0.13)	41.21 (0.15)	9.41 (0.12)
Lower Wyke Moor	0.80 (0.10)	5.61 (0.04)	5.23 (0.16)	48.30 (0.17)	9.76 (0.15)
Dairy South & Dairy Corner	0.89 (0.07)	5.56 (0.04)	5.99 (0.10)	52.55 (0.20)	11.75 (0.12)
Dairy East	0.91 (0.05)	5.40 (0.03)	6.25 (0.06)	55.12 (0.08)	11.99 (0.05)
Longlands North	0.91 (0.13)	5.53 (0.01)	4.27 (0.07)	31.24 (0.04)	6.78 (0.11)
PER FARMLET					
FARMLET A (n = 84)	0.91 (0.10)	5.64 (0.07)	5.28 (0.20)	43.41 (0.22)	10.04 (0.20)
FARMLET B (n = 81)	0.92 (0.09)	5.68 (0.05)	5.59 (0.13)	48.04 (0.16)	10.48 (0.20)
FARMLET C (n = 85)	0.86 (0.09)	5.49 (0.05)	5.28 (0.18)	46.65 (0.22)	10.33 (0.18)

[*]Catchment = Fields that are hydrologically isolated to measure runoff flow and quality

Conclusions

Catchments varied in respect of the parameters surveyed. Fields used previously for dairying were generally richer in nutrients. The means and coefficient of variation confirm that the allocation of catchments to each farmlet gave an even range of soil quality to each of the three farmlets which will in future have different land use treatments.

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"Virtual grassland": an individual-based model to deal with grassland community dynamics under fluctuating water and nitrogen availability

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Abstract

The "Virtual grassland" model is being developed to provide herbaceous grass and legume plant models and coupling methods with soil and light transfer modules on the OpenAlea platform. It aims at simulating the effects of competition and facilitation for light, water and nitrogen acquisition at the population and community levels in grasslands. Preliminary assessments of the coupled models suggest good qualitative behaviour with respect to light, defoliation and water responses. Quantitative model assessment is still ongoing.

Keywords: architecture, grass, legume, community, dynamics, water stress

Introduction

Multi-specific grasslands are a major source of forages worldwide. Their agricultural use-value depends on the structure of their canopy and on their botanical composition. Both determine the quantity and the quality of the biomass harvested by grazing or mowing. In temperate grasslands, perennial grasses and forage legumes dominate the floristic composition of the grasslands and are they generally intended to be grown in mixtures because of their agronomic (feeding value) and ecological (resource capture and use) complementarities (Louarn *et al.*, 2010). An appropriate species balance is, however, difficult to maintain in such non-equilibrium communities. The proportion of forage legumes fluctuates from year-to-year, within a year between harvests, and even within a single growth period as a result of species interactions and management. In spite of this agronomic significance, most grassland models developed so far ignore plant-plant interactions and do not simulate the community dynamics of grasslands. Efforts have been done mainly on white clover based mixtures for which a gain of understanding of coexistence conditions was achieved thanks to individual-based models (Schwinning and Parsons, 1996; Soussana and Oliveira Machado, 2000). However, these predictions may present a limited interest for other grass-legume combinations. Indeed, white clover has rather an atypical colonization strategy and persistence habit among temperate forage legumes. The exposition of shoot apex to defoliation and the lack of an efficient vegetative reproduction in most tap-rooted forage legumes are known to affect their population dynamic and could modify the impact that legumes have on nutrient cycling. Furthermore, for white clover, being grown predominantly in areas with limited water stress, water shortage effects have not been included in these models. In order to deal with these questions, the "Virtual grassland" model at (http://openalea.gforge.inria.fr/wiki/doku.php?id=packages:ecophysio:grassland) is being developed under the open source OpenAlea modelling platform (Pradal *et al.*, 2009) to simulate the architecture of various grass and legume species and predict the effects of plant interactions for light, water and nitrogen at the population and community levels.

Materials and methods

The "Virtual grassland" model provides:
- i) Two generic plant models accounting respectively, for grass (L-grass; Verdenal *et al.*, 2008) and legume (L-egume) morphogenesis. These models simulate 3D plant architecture above-

and below-ground and provide a central data structure describing plant structure from organ to community scales (MTG);

- ii) Coupling methods (in the form of OpenAlea nodes) between the MTG central data structure for plant architecture and environmental models to simulate competition for resources above- (light) and belowground (water and soil N). The light transfer (Sinoquet *et al.*, 2001), gas exchange (Prieto *et al.*, 2012) and soil models (adaptation of STICS soil module; Brisson *et al.*, 2008) are available through the Openalea platform and were reused from previously published models.

- iii) A graphical user interface in the form of a dataflow enabling to manage model input parameters and visualize simulation results (Figure 1).

The two plant models are based on the L-system formalism (L-py software, Boudon *et al.*, 2012) and aim at capturing variation in morphogenesis and C/N metabolism specific to each functional group. In grasses for instance, differences in the self-regulation of leaf growth, tiller development and primary root initiation enable to account for growth habit ranging from long-leaf forage species/cultivars to short-leaf turfs. Similarly, in the legume functional group, different growth habit in terms of shoot growth (erect/prostrate), branching, and ability to develop adventitious roots/shoots enable to distinguish between colonization strategies ranging from herbs perennating by formation of a single taproot (such as alfalfa or red clover) to clonal patches resulting from rhizome spreading (creeping lucerne) or rooted shoots at the soil surface (white clover). Dry matter accumulation, water and N requirements of each plant in a community are assumed driven by light interception.
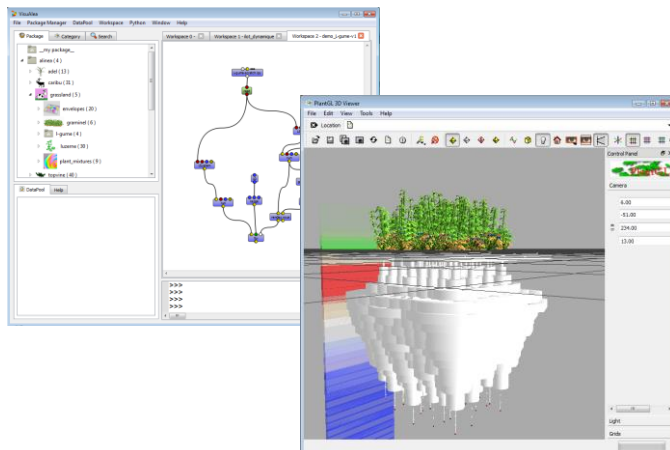
Figure 1. Graphical user interface and 3D plants illustrating an alfalfa plant population growing in a drying soil.

Results and discussion

The coupled soil-plant-atmosphere model is currently being assessed for both the behaviour of isolated plants (Figure 2), and the dynamic of plant populations in pure stands and in binary species mixtures. Parameters for morphogenesis were identified on contrasting alfalfa and perennial ryegrass cultivars. Morphogenetic response to light competition, defoliation and water stress were shown to be qualitatively consistent. Increased light competition resulted in reduced tillering/branching (and overall plant leaf area development), reduced root elongation rate (and root volume) and decreased root length density. An interaction between shoot morphogenesis (defining light competition ability) and defoliation regime existed for the two functional groups, morphotypes with short leaves (grass) / procumbent shoots (legume) being relatively favoured by more intense defoliations. Soil water availability affected first local root development and then plant water status, shoot growth and C assimilation/partitioning (Figure

2). Sensitivity to water stress was shown to be strongly dependent to developmental stages, and in particular to the rooting depth at the beginning of the stress.
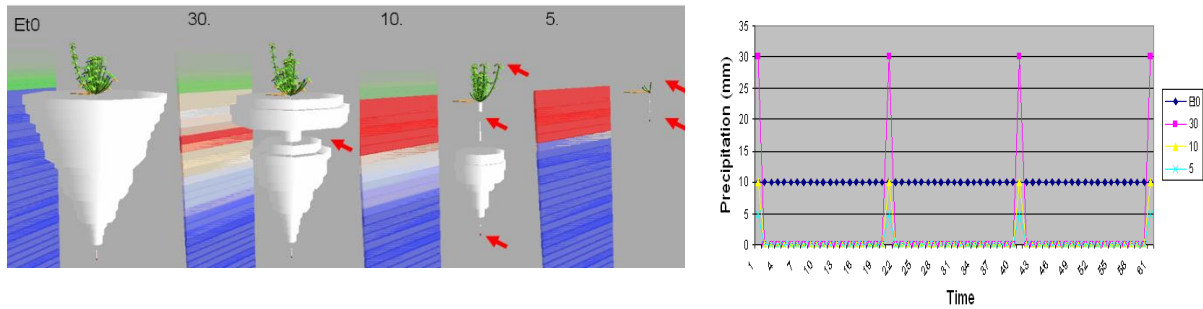
Figure 2. Illustration of the effect of four water regimes on the shoot and root morphogenesis of an isolated alfalfa plant.

Quantitative model assessment is still ongoing and implies the comparisons of population productivity, size structure, and N content between simulated and experimental stands under various conditions of light and water availability.

Conclusion

In its present state, the "Virtual grassland" model intends to provide an integrated framework for analyzing trade-offs between traits in multispecific grasslands and for helping in the identification of rules to formulate the composition of mixtures, considering interactions with both biotic and abiotic environments. Ultimately, it may also contribute to predict the effects of plant interactions for light, water and nitrogen on grassland community dynamics.

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Towards a new potassium fertilization recommendation in the Netherlands

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Abstract

The current potassium (K) recommendation in the Netherlands has been based on experiments from the past. The plots in those experiments were harvested at high yields and high N levels. Due to lower annual N levels and new soil extraction methods, the K fertilization recommendation should now be adapted. To obtain new information, in 2011 and 2012 a two-year grass cutting experiment on peat, sand and clay was set up with three N × eight K levels. The results show there is a need for about 50 kg K ha^{-1} for the first cut, and for every following cut the rate should be based on the expected amount of K-export in the grass. The effect of the first K application is still present in later cuts, but later cuts should also receive K fertilization based on expected K export by the yield. There is no interaction between N and K fertilization on the DM yield, but there is an interaction effect on K content. High K fertilization in combination with high N levels will lead to a higher K content of the grass.

Keywords: potassium, K-fertilization recommendation, DM yield, N×K interaction, K-content

Introduction

The common potassium (K) recommendation is based on research from 1930-1960, from experiments with high nitrogen (N) levels ('t Hart and van der Pauw, 1942). The recommendation was derived from data obtained at a harvest level of 4-6 t DM ha^{-1}. In present grassland management harvest took place at 1.5 (grazed plots) to 4 ton DM ha^{-1}. Recently, new extraction methods for K in the soil have become available, which will probably lead to an adapted K recommendation. Due to limited N fertilization, an interaction between lower N levels, K fertilization on DM yield, and K content is also expected. To collect data to support the adaptation, two parallel experiments were set up. One field experiment was set up to test the effect of different K-fertilization strategies in combination with two N levels. This experiment is described in this article. The main objective is to get information about the K response and the N × K interaction on DM yield and K content of the grass. (Another parallel experiment collects data from several small field experiments to analyse the relation between different soil types, soil parameters and K fertilization on the DM yield: this will be described in a separate report (Bussink *et. al*., in prep)). The outcome of both field experiments will provide new insights for an adapted potassium fertilization recommendation.

Material and methods

In 2011 and 2012 a field experiment was set up in the Netherlands on three soil types: peat, sand and young marine clay. Five cuts of grass were harvested in both years. The first cut was at two yield levels: about 1700 kg DM ha^{-1} and about 3500 kg DM ha^{-1}. The following four cuts were harvested around every 4-5 weeks. Two N levels (180 and 360 kg N ha^{-1} year^{-1}) and a control (no N fertilizer) were combined with eight K strategies. The N and K fertilization is given in Table 1.

The experiment was set up with two replicates, consisting of 3N × 8K = 24 plots on all soil types. The cuts were mown with a special mower for experimental research. The fresh yield was weighed and a sample was dried at 103^{0}C to calculate the DM yield. The differences between the DM yield on the plots were statistically analysed using residual maximum likelihood (REML: Harville, 1977) with the Genstat program (15th edition). The year effect was set as a random effect in the REML analysis.

Table 1. Nitrogen (N) and Potassium (K) fertilization (kg ha^{-1}) per cut and total

1	NK / cut no	2	3	4	5	Total
0	N0	0	0	0	0	0
60	N1	40	40	20	20	180
120	N2	80	80	40	40	360
0	K0	0	0	0	0	0
50	K1	0	0	0	0	50
50	K2	33	33	33	33	183
50	K3	66	66	66	66	315
100	K4	100	100	100	100	500
149	K5	0	0	0	0	149
149	K6	33	33	33	33	282
149	K7	66	66	66	66	415

Results and discussion

As expected, there was a strong ($P <0.001$) N response, which interacted with the soil type (the strongest effect on clay). There also was a significant overall K response ($P <0.001$) which also interacted with the soil type (hardly any effect on clay). There was no interaction effect on the DM yield between the N and the K application. This contrasts with the results of Widdowson *et al.* (1966), who found an interaction at the highest N level (314 kg N) in their experiment, although there was no interaction at N levels of 209 and 105 kg N ha^{-1} year^{-1}. Wolton *et al.* (1968) also found an interaction effect of N×K, but there was a large variation and the effect was not consistent. They saw no effect of K fertilization when no N was applied. This was is in contrast to the results of our experiment. We found K response at all N levels, including control plots without N fertilization. The effect of K fertilization on DM yield per cut was significant ($P<0.001$). This means that if a higher yield per cut is required, K application should be higher.
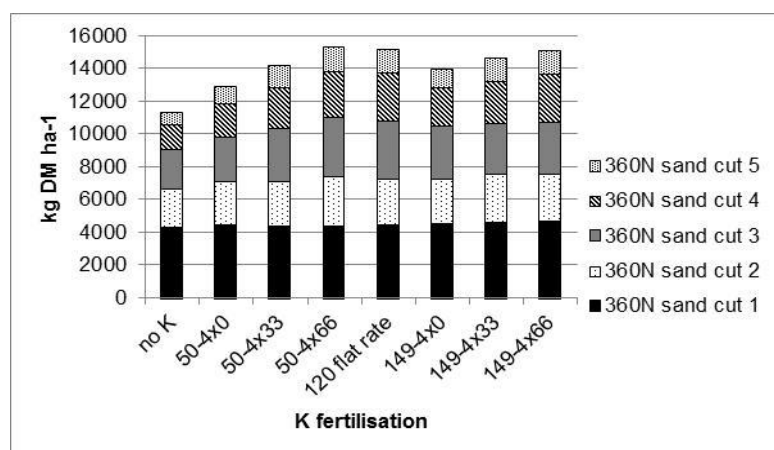
Figure 1. DM yield per cut and annual at eight K-fertilization strategies on sand and 360 kg N ha^{-1}

Figure 1 represents the results as an average over 2011 and 2012 on the sand location and for an N application of 360 kg N ha^{-1} year^{-1}. On the peat location similar results were found. On sand and peat an application of 50 kg K ha^{-1} is recommendable. A noticeable conclusion is that

the effect of K application in spring will have a (residual) effect on DM yield in later cuts. This effect can be seen comparing 149-4x0 K with the control without K. Not only in the first cut was DM yield higher, but also in later cuts that were not fertilized with K there was a higher yield. On peat and sand soils, later cuts respond significantly and positively to K application, which can be seen by comparing 50-4x40 with 504x66; the last strategy results in a higher annual DM yield. It seems that there is an upper threshold (maximum K application); a higher application did not result in a higher yield, but only in a higher K content. The effect of K fertilization on young marine clay was minimal, due to the type of soil. There was a slight difference (on average over all the plots and years, 500 kg) in DM yield in both years, but the effects were the same in 2011 and 2012. There is a significant effect of K fertilization on the first cut at all three soil types. The results of the experiment showed a higher K content in the grass when higher applications of K were given. High K contents should be avoided because of the risk of grass tetany (hypomagnesaemia) and too high K-applications also lead to losses. Based on this experiment (and the parallel experiment), an adapted K fertilization recommendation should be followed up.

Conclusions

An application of 50 kg K ha^{-1} for the first cut proved to be the most suitable recommendation. Due to the parallel experiment, a further differentiation will need to be made, that takes account of pH, soil type and K content (Bussink *et al.*, in prep). After the first cut, some K fertilization is recommended, although a residual effect of the K applied in spring was recorded. The application should be equal to the expected amount of K exported in the yield. No interaction between K and N application was found on the DM yield, but there was an interaction effect on the K content of the grass. Higher applications than the expected amount of K exported by the crop should be avoided.

Acknowledgements

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Increasing perennial ryegrass (*Lolium perenne*) yields using commercial bio-inocula on a phosphate-limited soil

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Abstract

Bio-inocula are seen as a potential way of increasing crop yields and the availability of plant nutrients, principally phosphorus – a major limiting nutrient. Bio-inocula contain a variety of plant growth promoting micro-organisms (PGPM) such as phosphate solubilizing bacteria and mycorrhizal fungi. Although many commercial products contain multiple strains of fungi and bacteria, the possibility of interspecific competition between fungal components could reduce inoculum effectiveness. This study aimed to examine this and the effect of commercial inocula on the yield of grass (*Lolium perenne*) grown on a phosphate-limited soil. Bacterial inocula significantly increased dry matter yield (3.51 t ha^{-1}) over controls. While not significant, grass treated with the mixed fungal treatment showed highest phosphate use-efficiency (0.47 g mg P^{-1}). The single fungal treatment had the highest root colonization (93.3% of roots colonized). Overall soil fungal diversity and evenness showed no significant differences across treatments. While initial results suggest some interspecific competition between fungal components of mixed inocula, further analysis is required to establish if this impacts on grass yields.

Introduction

Increased agricultural production will be required to meet global demand for feed, fibre, bioenergy and food. This will require increased inputs of key limiting nutrients such as phosphorus (P), and/or better utilization of P sources applied and existing soil P reserves (Cordell and White, 2011). Soil micro-organisms improve plant nutrient availability *(Barea et al.*, 2002) through processes of mineralization and solubilization of organic and inorganic soil phosphate, respectively. Identification of specific phosphate mobilizing fungi and bacteria has been exploited commercially through the development of bio-inocula.

Commercial inocula use multiple species and genera to help overcome host fungal specificity and improve economic viability, e.g. *Lolium perenne* has been shown to be dominated by the *glomus* genera (Gollotte *et al.*, 2004). Co-inoculation with different arbuscular mycorrhizal fungi (AMF) species is generally thought to be more effective due to functional complementarity within taxa (Koide, 2000), reducing plant investment costs to acquire P. However, functional redundancy may lead to negative effects between fungal taxons (Hepper *et al.*, 1988) and growth depressions have been observed when multiple species were added (Mickelson and Kaeppler, 2005).

The aim of this study was to establish the potential yield gains using commercial fungal inocula on a low-P soil; the use of fungal inocula in combination with low soluble phosphate fertilizer; and explore potential interspecific competition between fungal components of a mixed bio-inoculum.

Methods

A field trial was established at Bangor University's Henfaes Research Station in July 2013. Soil phosphate was measured at ~ 8mg kg^{-1}, placing the soil at Olsen extractable P index of 0. A commercial bacterial suspension (Bac) (GlensideGroup, West Lothian), commercial fungal consortia of 5 fungal species (MF) (*Glomus* SP., GlensideGroup) and a single fungal species

(SF) (*Glomus intraradices* BEG 72, Plantworks Ltd. Kent) were applied to replicated ($n = 9$) trial plots (1.8 m × 2 m) laid out in a replicated control strip design. Applications were made at ten times the manufacturer's recommendations, to improve subsequent detection rates (recommended application rates of MF and SF were 1 kg ha^{-1}). *Lolium perenne* seeds were dressed with each treatment and applied using a seed drill (34 kg ha^{-1}). The bacterial suspension (250 ml ha^{-1}) was diluted with water (150 L ha^{-1}) and applied with a hand sprayer. The phosphate treatments were applied to each strip as three treatments of TSP, RP and No phosphate ($n = 3$). N, K and phosphate treatments were applied in the spring (March 2014) as per RB209 recommendations (DEFRA, 2013) for a P index soil of 0.

The first cut biomass, 8 weeks after seeding, was measured for yield (t ha^{-1}), phosphate content (PO$_4$-P mg kg^{-1}) and fungal root colonization (Trouvelot *et al*., 1986) quantifying frequency (M_f) and intensity (M_i). Increased phosphate acquisition was investigated using a low and high soluble phosphate source (rock phosphate and triple super phosphate respectively). PUE (a measure of dry matter forage per unit phosphate acquired within the above ground biomass), was calculated and used to determine if bio-inocula improved phosphate acquisition. Ion Torrent DNA sequencing was used to assess any changes in biological communities after fertilization.

Soil samples taken ten days after seeding were frozen (-80 °C), after freeze-dried samples were ground (< 1 mm). DNA extractions were conducted using the MoBio Laboratories (Solana, CA, USA) PowerSoil DNA extraction kit. An Ion Torrent PGM^{TM} was used to sequence the extract. Sequences were clustered to 97% similarity using USEARCH and identified using the Ribosomal Database Project (RDP). A spreadsheet of phyla and quantities was produced for analysis, and used to calculate Shannon diversity index and equitability index.

Results and discussion

Results indicated yield gains with bio-inocula. The bacterial inocula significantly increased yield over the control, likely due to the nitrogen fixing species it contained, as the site received no N. The PUE of the bacterial inocula was similar to that of the other treatments, suggesting that the increased growth was not due to increased P acquisition but another limiting factor. Root colonization did increase with treatments; interestingly, the SF inocula colonized the roots more often and more intensely. This could be an indication of the potential interspecific competition within inocula formulation, the SF yielding ~20% more than the MF. That said, the PUE of the MF treatment was the highest of the 3 treatments and it is possible that this will factor in any potential yield gains after the phosphate treatments are applied. DNA analysis showed no real changes in diversity or evenness; however, soil samples were taken 1 week after seeding and, as such, any microbial additions may not have had time to establish. Soil samples 12 weeks after seeding may be more informative. Furthermore, the database is currently being refined to reduce the number of unidentified species. Therefore, future proposed analysis may reveal significant fungal community shifts, which the colonization and PUE data hinted at.

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Long-term effects of extensification regimes on soil and botanical characteristics of improved upland grasslands

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Abstract

To reveal key factors necessary for restoring previously intensively managed grasslands a long-term extensification experiment was established in Wales in 1994. The treatments were: sheep grazing (± lime); hay cut only (± lime); hay cut and aftermath sheep grazing (± lime); and sheep grazing plus fertilizer and lime application as a control. Nutrient concentrations in the soil together with plant species composition were assessed in 2012. The treatments which received lime had a higher pH and plant-available concentrations of Ca and Mg in the soil, whereas higher plant-available concentrations of P, K and organic C were recorded in the control treatment fertilized by NPK. Management was a key driver in terms of diversity of vascular plant species. Multivariate analysis identified three groups of treatments with similar plant species composition: control and grazed treatments as the first group; hay cut treatments as the second group; and hay cut plus aftermath grazing as the third group. Lime application had relatively little effect on plant species composition, but it decreased the hemiparasitic species *Rhinanthus minor* which can initiate further steps within the extensification process. The most effective management for grassland biodiversity restoration was hay cutting with aftermath grazing.

Keywords: extensification, grazing, cutting, nutrients, vegetation

Introduction

Long-term application of NPK fertilizers increases plant productivity and soil nutrient concentrations but also reduces plant species richness, and this can result in the dominance of largely species-poor grasslands with little conservation value. High concentrations of plant-available P in the soil are particularly associated with low species richness and dominance of highly productive species (Tallowin and Smith, 2001). The restoration of species-rich grassland on previously agriculturally improved grasslands has a number of specific abiotic and biotic constraints, including high nutrient contents in the soil and a lack of desirable species. In order to achieve restoration of species-rich grassland it is necessary to identify key stages and likely timescales, as well as practical management techniques to be undertaken. To explore these issues further, a long-term extensification experiment testing different management regimes was set up at the Pwllpeiran Upland Research Centre in 1994 (the Brignant plots) (Defra, 2006). Preliminary results showed that the cessation of fertilizer use and imposing of extensive management practices allowed moderately species-rich grassland communities to develop within a 10-year timescale. In this study we analyse extensification management effects on soil nutrients and plant species composition after 18 years of treatment imposition.

Materials and methods

The experiment was established on previously improved and consequently intensively managed pasture situated within the Cambrian Mountains at an altitude of 310 m a.s.l. Grass species dominated in the sward in 1994; particularly *Lolium perenne*, at 58% cover. Soils at the site are free-draining typical brown podzolic soils, with the area receiving a mean annual rainfall of 1770 mm. The treatments applied were: i) sheep grazing with fertilizers and lime application as control (CO); ii) hay cutting followed by aftermath sheep grazing, with (HGL+) and without (HGL-) lime application; iii) hay cutting only, with (HL+) and without (HL-) lime application; iv) sheep grazing, with (GL+) and without (GL-) lime application. The control treatment was a continuation of the previous relatively intensive management practice of an annual application of 60 kg N fertilizer and 30 kg P fertilizer ha^{-1}. The lime treatments received a single application of lime in 1998 with the intention of maintaining a soil pH of 6.0. Treatments are replicated three times in a randomized block design with individual plots 0.08 ha (hay cut only) or 0.15 ha (grazed) in size.

Fifteen individual soil cores were taken to a depth of 0-7.5 cm from randomly located areas from within each plot in July 2012. Plant-available Ca, K, Mg and P were extracted by the Mehlich III method (Mehlich, 1984) and then determined by ICP-OES. Total N was analysed by the Kjeldahl method and organic C by means of colorimetry. Visual percentage cover of vascular plant species was estimated in 10 randomly located (0.4 m^{-2}) quadrats per plot in July 2012. Redundancy analysis (RDA) in the CANOCO 4.56 program was used to evaluate multivariate vegetation and soil nutrient data.

Results and discussion

Management treatment explained 35.5% ($P<0.001$) and 60.6 % ($P<0.001$) of the variability of the soil nutrient content in the first and all axes, respectively. The first axis of RDA displayed a gradient of pH in the soil (Fig. 1a). The treatments which received lime application in 1996 had higher pH and plant-available content of Ca and Mg in the soil, whereas higher plant-available contents of P, K and organic C were found in the CO treatment fertilized by NPK. These results concur with studies which have shown that residual effects of former fertilizer treatments can be identified even when applications were short term (Pavlů *et al*., 2011).

Treatment effects explained 59.8% ($P<0.007$) and 81.1% ($P<0.001$) of the variability in plant species composition in the first axis, and all axes, respectively. RDA analysis of the vegetation data revealed that the first axis displayed a gradient of the defoliation management (Figure 1b). Three groups of treatments with similar plant species composition were recognized on the ordination diagram based on RDA analysis of data collected in 2012: CO, GL+ and GL- treatments as the first group; HL+ and HL- treatments as the second group; and HGL+ and HGL- as the third group. The first group was species poor (number of species <8) and promoted especially grasses that are adapted to frequent defoliation (e.g. *Poa pratensis, Agrostis capillaris, Lolium perenne, Festuca rubra*) and the nitrophilous species *Urtica dioica*. In the second group the species sensitive to defoliation were more abundant (e.g. *Holcus mollis, Alopecurus pratensis, Cirsium palustre*). The third group was the most species rich (number of species >13) and included forbs species adapted to frequent defoliation (e.g. *Hypochoeris radicata, Potentila erecta, Taraxacum* sp., *Crepis capillaris*) but which need hay management to enable seed reproduction. *Rhinanthus minor* was the only species suppressed by previous lime application in extensively managed treatments. This species had not been recorded previously within the plots and is important as it can suppress existing vegetation and consequently accelerate the introduction of the other competitively weak species occurring in the surrounding areas. Overall it appears that management treatment applied was the key driver

in terms of diversity of vascular plant species. Similar results were found in extensification experiments in Germany (Hejcman *et al.*, 2010; Pavlů *et al.*, 2011).
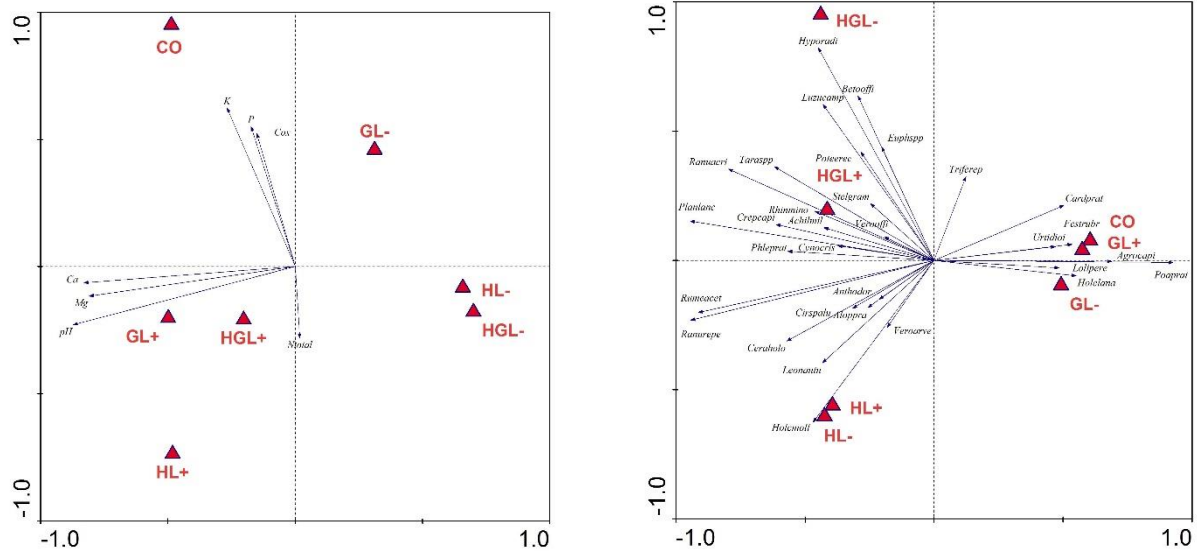
Figure 1. Ordination diagram showing the results of RDA analysis: a) nutrient content in the soil (left); b) plant species composition (right). For treatment abbreviations see Materials and methods section. Species abbreviations are based on first four letters of genus and first four letters of species name.

Conclusion

Although former lime and NPK fertilizer applications were still detectable in the soil, their effect on botanical composition was relatively small. The key effect on plant species composition was the management treatment applied. The most effective management for grassland biodiversity restoration was hay cutting with aftermath grazing.

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How do pre-sowing disturbance and post-establishment management affect restoration progress in ex-arable calcareous grassland?

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Abstract

Restoration of semi-natural calcareous grassland needs to overcome dispersal limitation and microsite limitation. The effectiveness of sowing a mix of ten target species in combination with various pre-sowing disturbance techniques and post-establishment management regimes in overcoming these limitations, and in promoting vegetation change towards the restoration target was tested in a field experiment. Our results illustrate the importance of bare ground for introducing target species of calcareous grassland into species-poor grassland, and the slowness of colonisation by additional target species from outside even when a site is situated close to high-quality grassland that could serve as source for additional target species.

Keywords: bare ground, compositional similarity, ecological restoration, target species

Introduction

Species-rich lowland calcareous grassland in the UK has markedly declined in extent over the past century. To reverse this decline, efforts are made to restore such grassland through grassland creation and the diversification of species-poor grassland (Walker *et al*., 2004). Grassland restoration has to overcome two key limitations (Bakker and Berendse, 1999). Dispersal limitation occurs because unassisted re-colonization by target species tends to be slow, and depends on local presence of existing high-quality grassland. Microsite limitation, a lack of small-scale sites suitable for seed germination and seedling establishment, can be a serious obstacle because soil fertility is often considerably raised, and existing vegetation tends to be highly productive and dense. Generalist grassland species which tend to be competitive and capable of rapid clonal spread usually perform better under such conditions than specialist species that are adapted to low soil fertility and that depend more strongly for regeneration on seedling establishment in seasonally available gaps (Pywell *et al*., 2003). We investigated in a 4-year field experiment how conditions for introduction and short-term persistence of specialist species of calcareous grassland can be provided during restoration. Based on annual vegetation surveys, and using reference data from local calcareous grassland, this paper compares restoration progress among various experimental treatments.

Materials and methods

A split-plot experiment was set up in spring 2008 on species-poor grassland in the Pegsdon Hills (51° 57' N 0° 23' W) at the NE edge of the Chiltern Hills, SE England. The grassland was created in 1993 on previously arable land by over-sowing with a low-diversity grass mix and subsequent sheep grazing. In 2008, vegetation at the site mainly consisted of *Arrhenatherum elatius*, *Agrostis stolonifera*, *Poa trivialis*, *Medicago lupulina* and *Trifolium repens*. Close to the restoration site there were areas of species-rich calcareous grassland. Twelve main plots of 75 m × 50 m were set up in four replicate blocks to compare different management regimes,

and at the centre of each plot, a grid of 5 m × 5 m cells was laid out to implement different types of pre-sowing disturbance at the sub-plot level. Allowing for guard rows between experimental sub-plots, disturbance treatments were assigned to grid cells at random. Prior to disturbance, herbage was cut and removed. Disturbance treatments were (1) undisturbed control; (2) band-spraying with glyphosate to kill c. 50% of the vegetation; (3) power harrowing to create c. 70-80% bare ground; and (4) 'ridge-and-furrow', i.e. creation of a 1.5 m wide × 0.4 m high ridge by two-directional ploughing. After disturbance, all experimental sub-plots were over-sown on 17 May 2008 with a seed mixture containing ten species of calcareous grassland (*Bromopsis erecta*, *Campanula glomerata*, *Carex flacca*, *Filipendula vulgaris*, *Helianthemum nummularium*, *Hippocrepis comosa*, *Pimpinella saxifraga*, *Stachys officinalis*, *Succisa pratensis* and *Thymus pulegioides*). Sowing rates varied among species, ranging from 30 to 120 seeds m^{-2}. In 2008, the site was uniformly managed with a hay-cut in late July and aftermath cattle grazing. From 2009 onwards, three management regimes were implemented at main-plot level: (1) summer hay-cut followed by autumn cattle grazing; (2) spring grazing with sheep, followed by summer hay-cut and autumn cattle grazing; and (3) spring grazing with sheep, followed by summer and autumn cattle grazing. From 2009 onwards, cover of vascular plant species was recorded annually before the summer hay-cut in two permanent 1 m x 1 m quadrats per sub-plot. Prior to statistical analyses, data from both quadrats was averaged. Quadrat data (*n* = 39) from eight calcareous grasslands located within 25 km from our site was used to define the restoration target. To explore vegetation change in across treatments in relation to this target, Non-metric Multidimensional Scaling (NMDS) based on the Relative Sorensen distance measure was carried out using PC-ORD V6.08 (McCune and Mefford, 2011). Similarity of vegetation in experimental treatments with target vegetation was further investigated with a repeated-measures linear mixed model, using PROC MIXED in SAS V9.3 (SAS, 2011). For this analysis, Relative Sorensen distances between each sub-plot in each year and quadrats from reference grassland were converted into percentage similarities, and within each year, the 39 similarity values per experimental sub-plot were averaged and log-transformed. Management regime, pre-sowing disturbance, year, and the various interactions between these entered the analysis as fixed factors, and year as repeated-measures factor. Blocks and main plots nested within blocks were specified as random effects. A similar analysis was carried out for total cover data of sown species, which was also log-transformed prior to analysis. The most appropriate covariance structure from a list including compound symmetric and various autoregressive structures was selected on the basis of the Akaike Information Criterion.

Results and discussion

According to the NMDS biplot (Figure 1), between 2009 and 2011, vegetation composition in experimental treatments has gradually become more similar to target grassland composition. Mixed-model analysis shows that rate of increase in similarity over time with the calcareous grassland target vegetation is significantly affected by management regime and initial disturbance (Figure 2A). This increase was more pronounced in ridge-and-furrow sub-plots and in harrowed sub-plots. Regarding management regimes, it was less pronounced under sheep grazing in spring in combination with summer and autumn cattle grazing. However, progress was generally slow, and after three years, even in the most successful treatment combinations, vegetation was only about 10% similar to the target. Increase in total cover of sown species (Figure 2B) varied similarly between treatments, suggesting that the observed increase in similarity to target community composition was largely driven by changes in sown species cover. A closer look at the vegetation data confirms that levels of colonisation by additional species were very low, although there were shifts in abundance of unsown resident

species. Slow colonisation in spite of proximity of high-quality source grassland was also found by Hutchings and Booth (1996).
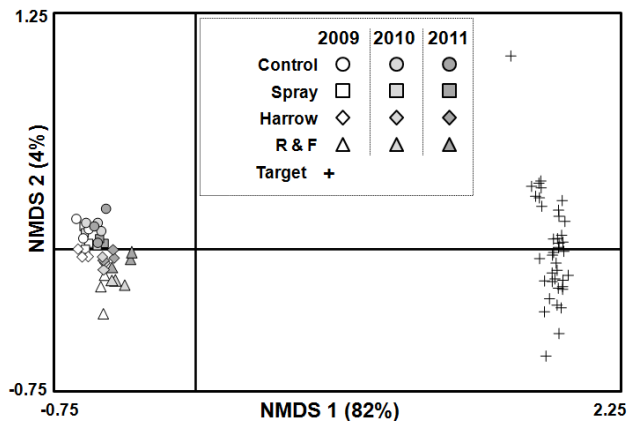
Figure 1. NMDS biplot of vegetation in experimental treatments 2009-2011 in relation to calcareous grassland (= target).
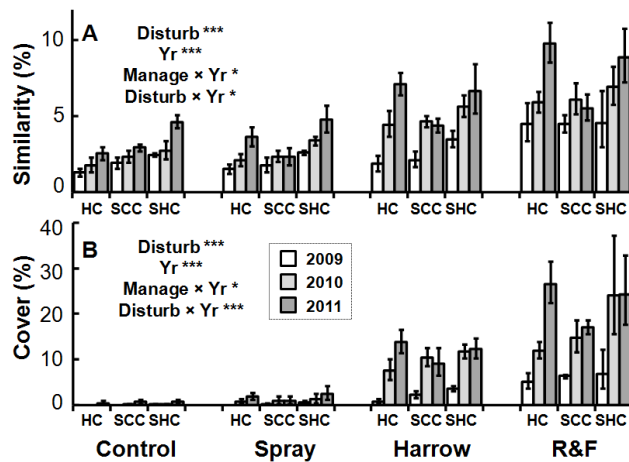
Figure 2A: Similarity of experimental treatment vegetation to target vegetation. Figure 2B: Total cover of sown species. (HC: hay cut + autumn cattle; SCC: spring sheep + hay cut + autumn cattle; SHC: spring sheep + hay cut + autumn cattle.)

Conclusion

Our results highlight the importance of bare-ground creation for successfully introducing late-successional target species of calcareous grassland into species-poor existing swards. They also illustrate that colonization from nearby species-rich grassland is slow when unassisted, and that, in order to achieve reasonably fast restoration of species-rich calcareous grassland, higher levels of intervention may be required, involving either active introduction of a wider range of target species, which could take place in a staged manner (Pywell *et al.*, 2003), and/or grazing regimes targeted at assisting processes of species immigration from nearby species-rich source grassland (Poschlod *et al.*, 1998).

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Sown biodiverse pastures as a win-win approach to reverse the degradation of Mediterranean ecosystems

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Abstract

The system of 'sown biodiverse permanent pastures rich in legumes' started being developed in Portugal in the second half of the 1960s as an economically rational strategy to increase grassland productivity, by sowing mixes of up to 20 species/cultivars of legumes and grasses. Compared to natural pastures, the resulting semi-natural system provides higher yields of better quality pasture, allowing an increase of sustainable stocking rates, with environmental co-benefits. In this work we present a conceptual model of the environmental and economic effects of this system when compared with an alternative (natural pastures), complete with a summary of evidence supporting the claim that this system is an economic and ecological win-win solution that addresses many of the causes of land degradation in the Mediterranean while also recovering soil condition, ecosystem functions and services.

Keywords: biodiversity engineering, ecosystem restoration, carbon sequestration, semi-arid land, sustainable intensification, soil organic matter

Introduction

'Sown Biodiverse Permanent Pastures Rich in Legumes' (SBPPRL) is a system of engineered pastures that uses biodiversity as a leverage for pasture productivity. The main rationale behind this system is the introduction of species or varieties specific of arid and semi-arid regions, either absent or in lesser percentage in spontaneous grasslands (as, for example, some varieties of legumes), with the aim of establishing a functioning ecosystem with complementary ecological niches, and which is more productive than less-diverse grasslands. The higher productivity and feed quality of SBPPRL are paired by several other benefits to farmers, but also to society. SBPPRL have been described as a win-win solution (environmentally and economically) that combines the private interests of farmers with the public services provided, such as carbon sequestration through increases in soil organic matter (SOM), which in turn may help reverse the degradation of Mediterranean arid and semi-arid ecosystems. For example, the system won in 2013 the 'World You Like Challenge' of the European Commission (http://world-you-like.europa.eu/) for the best solutions to address climate change. It was described by the European Commissioner for Climate Action as 'a perfect example of how practical solutions for climate action can also save money and create jobs and growth'. Since 2009, the Portuguese Carbon Fund (a financial instrument created to help the country comply with Kyoto targets) has been supporting the installation and maintenance of SBPPRL through a system of payments for carbon sequestration. More than 50 000 ha were sown under this programme, which represents an estimated amount of 1.54 million t of sequestered CO_2 between 2008 and 2014. In addition, between 1996 and 2008 carbon

sequestration by SBPPRL was estimated as 3.5 million tons of CO_2, at an approximate rate of 5 t of CO_2 per hectare (Teixeira, 2010).

The baseline system

Grazing has been a central activity in the Mediterranean, shaping these ecosystems for millennia. Fire, clearing of shrubs and reducing forest density have been employed to maintain or reverse the process of vegetation succession (Bugalho *et al.*, 2011). Today, these Mediterranean ecosystems occupy vast areas where soils are shallow, poor in organic matter and nutrients, and much exposed to soil erosion (Van-Camp *et al.*, 2004). This marginal land has been used as grazing land to produce livestock, in what we designate here as natural pastures (NP). NP areas have historically been where SBPPRL are installed, and for comparison purposes we will consider them as the baseline alternative.

Ecological and economic effects of SBPPRL

The effects of SBPPRL relative to NP are shown in Figure 1, and can be qualitatively summed up as follows. Increased productivity in SBPPRL allows a sustainable increase in animal carrying capacity. Animals graze the plants, which have an annual life cycle. High plant productivity implies increased atmospheric carbon capture through photosynthesis. Part of the biomass produced is stored in soils due to the high density of yearly-renewed roots. Storage occurs in the form of non-labile (lower decomposition rates) soil organic carbon (SOC), which is part of the SOM pools. The increase of SOM improves soil nutrient availability and water holding capacity, thus increasing plant productivity and reducing surface runoff of water, which in turn decreases sediment loss and soil erosion. This effect is further strengthened by avoided soil mobilization due to seed bank persistence, which can last for more than 20 years in well managed pastures. In addition, decreasing water runoff and soil erosion contribute to reduce silting, eutrophication and contamination of surface waters outside the sown plot. Nitrogen fixation by legumes eliminates the need for synthetic nitrogen fertilizers, the production of which is highly energy demanding, and therefore responsible for high greenhouse gas emissions. Finally, the sustainable increase of the stocking rate and reduced fertilizer use, and the remuneration for ecosystem services, such as carbon sequestration, increase the economic viability of the farms while addressing both drivers of ecosystem degradation.

Quantitative data already exist for some of the causal links in Figure 1. The first experiment to assess the effects of SBPPRL gathered data from rainfed pastures (SBPPRL and NP) on eight farms from 2001 to 2005 (Teixeira, 2010; Teixeira *et al.*, 2011). Each plot's soil and landscape type was approximately homogeneous, in terms of soil and previous use (Teixeira *et al.*, 2011). Dry matter (DM) productivity is systematically 50 to 100% higher for SBPPRL, increasing every year and reaching 2-6 t DM per hectare (Teixeira, 2010). Average stocking rate is 1.0 Cattle Unit (1 CU ≈ 1 adult cow) per hectare and per year in SBPPRL and 0.43 CU ha^{-1} year^{-1} in NP. SOM concentration increases on average by 0.21 percent points (pp) per year in SBPPRL, during the first 10 years, and by 0.08 pp per year in NP (Teixeira *et al.*, 2011). The 0.21 pp increase in SOM is equivalent to the sequestration of 6.5 t of CO_2 ha^{-1} year^{-1} in the first 10 cm of topsoil and during 10 years after installation (Teixeira, 2010). The overall greenhouse gas (GHG) balance of SBPPRL, considering CO_2 emissions from limestone application to correct soil acidity, N_2O emissions from nitrogen cycle, CH_4 emissions from livestock and CO_2 sequestration by plants, is of 1.55-2.13 t of CO_2 ha^{-1} year^{-1} (Teixeira, 2010), which verifies its role as a carbon sink.
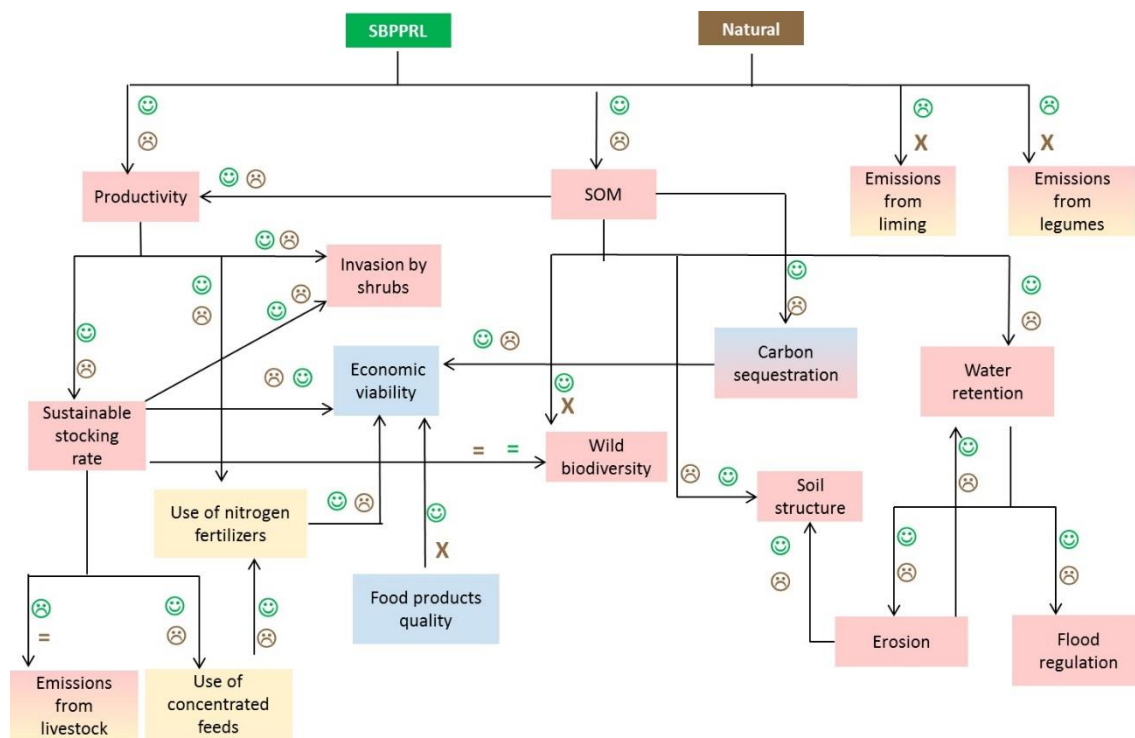
Figure 1. Relative effects of SBPPRL and NP. Arrows indicate causal relation. Green emoticons are the effects of SBPPRL and brown emoticons of NP. Direct ecologic effects (at the farm) are represented in pink, indirect effects in yellow and socioeconomic effects in blue. Two colour blends indicate two types of effect.

Conclusion

SBPPRL are sustainable semi-intensive systems for meat production applied in Portugal that maximize the bundle of services provided by grasslands, and contribute to counteract degradation drivers. SBPPRL provide better results than NP for most environmental performance indicators studied to date, while also delivering socioeconomic benefits.

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Fauna-flora relationships within improved upland grasslands managed under alternative extensification regimes

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Abstract

The maintenance of plant communities in grasslands depends on regular disturbances, but their sustainable management demands the integration of both floral and faunal components. This study tested the short and long-term impacts of sheep grazing and/or hay cutting (both with and without lime addition) on arthropod foliage fauna within Cambrian Mountain grasslands. Data were collected from replicate plots during the summer of 2013; twice before hay cutting and once just after. Fauna were sampled with sweep nets, and complementary data on sward height, flower numbers, and percentage of forbs and grasses were collected. Total fauna abundance was higher in grazed plots (as Symphypleona flourished there), while family richness was higher in swards managed for hay cuts. However, taxa-specific responses occurred. Certain Heteroptera abounded in grazed areas whereas Diptera or certain Coleoptera were linked to hay cut areas. Short-term effects reflected phenological changes (e.g. in Symphypleona or Cantharidae) and fauna reductions after hay cutting, when mostly Diptera remained. Fauna communities were associated with flower-rich and forb-dominated areas, although certain taxa favoured grazed and grass-dominated ones. Both hay cutting and hay cutting plus aftermath grazing were linked to higher levels of biodiversity by providing more suitable environmental conditions for the fauna.

Keywords: arthropoda, sheep grazing, hay cut, management

Introduction

Grassland ecosystems depend on regular disturbances to prevent vegetation succession and maintain a particular plant community, although whenever simultaneous sustainability is expected, the strategies must integrate both floral and faunal components (WallisDeVries *et al*., 2002). Common management strategies for improved grassland in the UK uplands include grazing and/or cutting, plus fertilization and lime addition. European grasslands agri-environment schemes encouraged modification of such strategies to arrest biodiversity loss, but their effectiveness has been challenged (Kleijn *et al*., 2003). The aim of the current study was to test the long-term and short-term effects of such management options on foliage arthropod fauna.

Materials and methods

The study was based on long-term plots at the Pwllpeiran Upland Research Centre. The replicated plots (known as the Brignant plots) were set up in 1994 on a previously improved pasture dominated by grass species in the Cambrian Mountain ESA. The experimental design consisted of a randomized block design with three blocks and four grassland management regimes imposed on individual plots 0.08 ha (hay cut only) or 0.15 ha (grazed) in size. The control (CO) was the continuation of normal intensive management (i.e. limed, fertilized and continually grazed by sheep). The treatments G (sheep grazing), H (hay cut) and HG (hay cut and aftermath grazing) represented different options for extensification, each one with (+) and without (-) lime addition. Fauna and flora data were recorded three times during the summer

of 2013: 18 July (S1), 16 August (S2) and 28 August (S3). The hay was harvested on 26 August. Foliage arthropods were captured using a sweep net along random transects (two or three per plot, depending on size); with fifty sweeps made per transect. Fauna were identified to order or family level (Coleoptera, Hemiptera and Araneae). Sward surface height, the percentage of forbs and grasses, and the number of flowers were also recorded for each plot at the time of sampling. Mixed-model ANOVA was used to test for treatment effects in the fauna and flora over time and linear ordination method redundancy analysis (RDA) to test the responses of arthropod communities to the management treatments and plant variables.

Results and discussion

A total of 62260 arthropods were collected and assigned to 13 orders. Symphypleona, Diptera, Hemiptera and Hymenoptera accounted for 33%, 23%, 21% and 14% of the catches respectively. Comparing the three periods, the total abundance changed over time and between treatments ($P < 0.001$). The catches in S2 were higher than in S1 and S3 ($P < 0.001$); with the lowest records in S3 reflecting the drastic decline in fauna on H and HG sites following the hay cut. Total fauna abundance was lower in G than in CO and H ($P < 0.01$) and lime addition had no effect on abundance and diversity. Focusing on S1 and S2, short-term (seasonal) and long-term (treatment effects) were observed. The total abundance was lower in G than in HG ($P < 0.1$), H and CO ($P < 0.05$). It increased in S2 as Symphypleona peaked in CO; in fact, this order was more abundant in CO than in the other treatments during both periods ($P < 0.05$). If Symphypleona was excluded from the analyses the global patterns were consistent along time: higher fauna catches in H and HG ($P < 0.05$) and G ($P=0.055$) than in CO. Taxa-specific responses were observed. Sap-sucking insects from Hemiptera abounded more in H than in HG and G ($P < 0.001$). Within this order, relevant families responded differently. Miridae were more abundant in H than in G and HG ($P < 0.001$), and Aphididae in H than in G ($P < 0.001$), whereas Cicadellidae peaked in G compared to H and HG ($P < 0.05$), and was also more abundant when lime was added ($P < 0.001$). Diptera flourished in HG ($P < 0.01$) and H ($P < 0.05$) compared to G. Within this order, a relevant group in pollination and pest control, Syrphidae, was clearly linked to HG and H sites than to the others ($P < 0.001$) whereas Tipulidae, a frequent food resource for birds, was indifferent to the treatments. The order Coleoptera was more abundant in H sites than in the rest ($P < 0.001$), largely due to the preference of flower visiting Cantharidae ($P < 0.001$) for these sites during S1. Araneae was globally indifferent to the treatments, although the catches of common inhabitants of flowers and leaves like Thomisidae peaked in H and HG compared to CO and G ($P < 0.001$ and $P < 0.01$ respectively), and also in sites with lime addition ($P < 0.01$), whereas agrobiont Linyphiidae did not differ between treatments. Family richness differed between treatments and was higher in H than in CO ($P < 0.05$) whereas Shannon index was higher in G than in CO ($P < 0.05$).

Regarding the differences between S2 and S3 (i.e. before and after hay cut), total abundance changed between periods ($P < 0.001$), increasing sharply in CO, maintaining in G and dropping in H and HG during S3 (with no differences between these treatments) compared to CO and G ($P < 0.001$). Fauna richness and Shannon index was reduced in hay cut sites (where mostly Diptera dominated) compared to grazed ones ($P < 0.001$), where no differences were observed. Regarding the importance of vegetation variables for the arthropod communities, the model which included all of these as environmental variables and the replicate blocks as covariables was significant ($P < 0.001$) when tested separately for each period. In the long term, arthropod communities were more commonly linked to taller, flower-rich areas with higher percentage of forbs, although certain groups were associated with grass-dominated areas (Figure1a). By contrast, after the hay cut, as vegetation was removed in the areas with a greater percentage of forbs (H and HG), fauna was linked mostly to grass-dominated sites (Figure 1b).
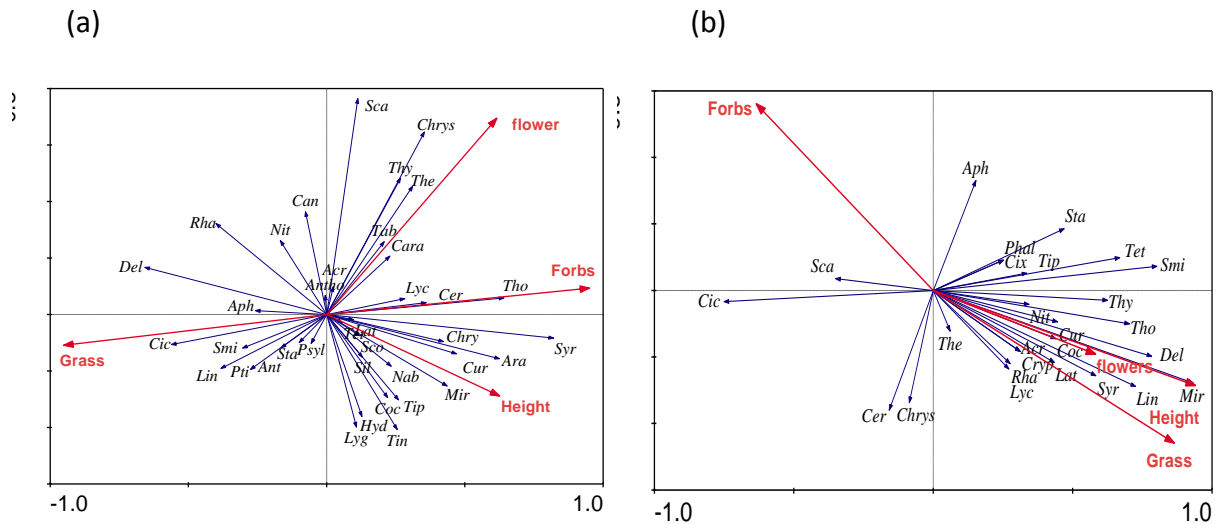
Figure 1: RDA analysis for flora-fauna relationships during the second (a) and third (b) periods. Blue lines correspond to the records of arthropod families and red ones to plant data. Standardized by sample norm and blocks included as covariables. Unrestricted permutations. 0.31 and 0.43 for all canonical axes in (a) and (b) respectively. Eigenvalues in axis one were 0.18 and 0.43 for (a) and (b) respectively.

Conclusions

Both short-term and long-term consequences of management options must be considered as they might lead to contrasting conclusions. In the long term, management for hay was linked to increased fauna richness through the provision of greater variability of plant resources, but in the short term, hay-cut areas suffered the highest fauna losses. Understanding the effect of land use on grassland communities contributes to the conservation of these often highly diverse habitats.

Acknowledgements

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Sewage sludge fertilization effects on *Q. rubra* and pasture production and flora biodiversity

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Abstract

Fertilization is usually applied to increase land productivity, but this technique also affects biodiversity in silvopastoral systems. This study aims at evaluating the effect of different doses of sewage sludge on tree growth, pasture production and biodiversity during three years, at eight years after establishment of a silvopastoral system with *Quercus rubra*. Improving soil fertility increases grassland biodiversity until tree canopy cover is not complete.

Keywords: agroforestry, sowing, afforestation, anaerobic digestion, composting, pelletization

Introduction

Fertilization is usually applied to increase land productivity, but this technique also affects biodiversity in a spatial and temporal scale in grasslands. The introduction of a tree in a silvopastoral system also modifies biodiversity as it initially generates heterogeneity (unshaded and shaded areas) but later on, when tree canopy completely covers the understorey, a new homogeneous situation is created. Broadleaf trees are generally used in silvopastoral systems, *Quercus rubra* being of high interest due to its high timber quality and tree growth rate. The combination of trees and grassland is usually more efficient in the uptake of nutrients from soil, the tree being a good tool to use and recycle nutrients applied to the understorey with fertilizers. The use of sewage sludge as fertilizer has been promoted in the EU in recent years due to the high nutrient content. Tree development, as well as soil fertility improvement, could modify the relationship among the different understorey species. This study aims at evaluating the effect of different doses of sewage sludge on tree growth, pasture production and biodiversity during three years, at eight years after the establishment of a silvopastoral system with *Q. rubra*.

Materials and methods

The experiment was established in Baltar, A Pastoriza (Lugo, Galicia, northwest Spain) at an altitude of 475 m above sea level. Pasture was sown with a mixture of *Dactylis glomerata* L. var. Artabro (12.5 kg ha⁻¹), *Lolium perenne* L. var. Brigantia (12.5 kg ha⁻¹) and *Trifolium repens* L. var. Huia (4 kg ha⁻¹) in December 2004. Plants of *Q. rubra* Franco were planted at a density of 434 trees ha⁻¹ after pasture sowing in 2001. The experimental design was a randomized complete block with three replicates and four treatments. Each experimental unit had an area of 368.64 m² and 25 trees planted with an arrangement of 5×5 stems with a distance between rows of 4.8 m, forming a perfect square. Treatments consisted of four doses of anaerobic sewage sludge meaning that 0, 100, 200 and 400 kg total N ha⁻¹ were applied in 2001, 2002 and 2003. Sewage sludge was applied superficially and the calculation of the required amounts was conducted according to the percentage of total N and dry matter contents (EPA, 1994) and taking into account the Spanish regulation (R.D 1310/1990) (BOE, 1990) regarding heavy metal concentration for sewage sludge application. Tree heights were measured with a graduated ruler in October 2008 and pasture production was determined by taking four samples of pasture per plot at random (0.3 × 0.3 m²) during the spring and winter from 2002 to 2008. In the laboratory, the pasture samples were dried (72 hours at 60ºC) and weighed to estimate dry matter production. Annual pasture production per year was calculated by summing the

consecutive harvests of the pasture production in that year. Before drying, the pasture samples were separated into the different species by hand. Data were analysed using ANOVA and differences between averages were shown by the LSD test, if ANOVA was significant. The statistical software package SAS (2001) was used for all analyses.

Results and discussion

Tree growth was significantly higher in treatments receiving 200 and 400 kg N total ha^{-1} than 0 or 100 kg N ha^{-1}. However, pasture production ha^{-1} was not modified during the three years, due to the lack of residual effect of fertilization. Higher doses of sewage sludge significantly increased pasture production in the first years of the experiment (Ferreiro-Domìnguez *et al.* 2011). Pasture production was reduced in the treatment with 200 kg N total ha^{-1}, probably explained because tree canopy cover was complete.
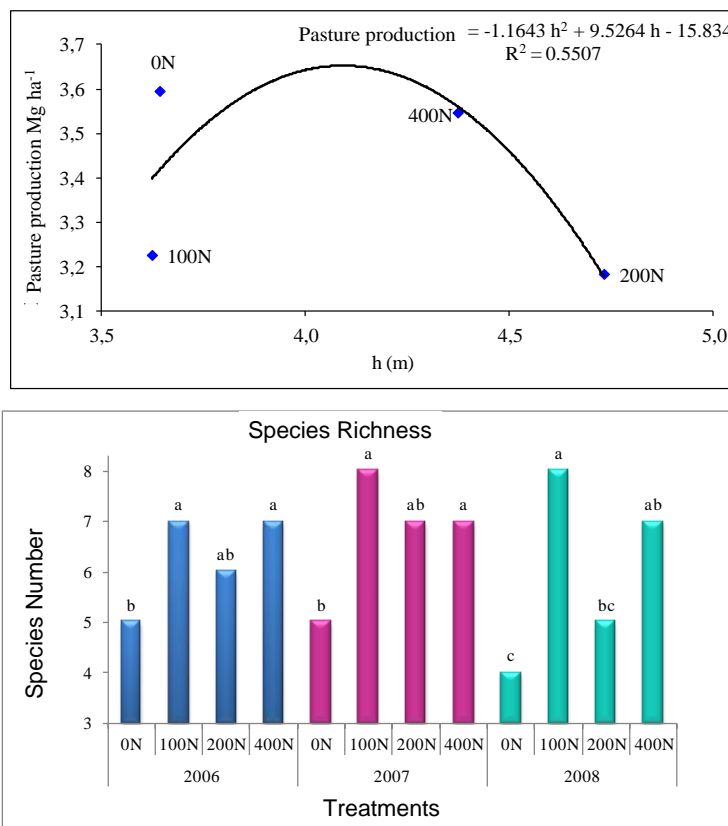
Figure 1. Tree height (m), annual pasture production (Mg ha^{-1}) relationship in 2008 is shown in the upper figure. Lower figure shows species richness per treatment Different letters indicate significant differences between treatments.
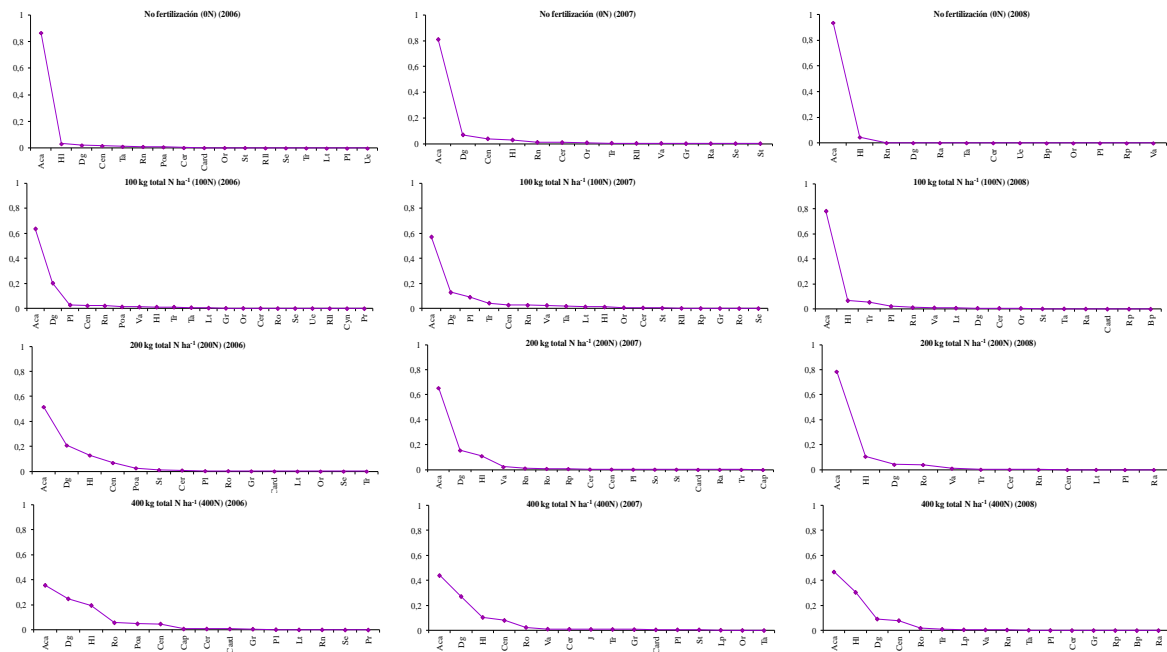
Figure 2. Abundance diagrams from 2006 to 2008 in the different treatments. Aca: *Agrostis capillaris* L., Bp: *Bellis perennis* L., Cap: *Capsella bursa pastoris* L., Card: *Cirsium arvense* L., Cen: *Centaurea limbata* Hoffmanns, Cer: *Cerastium glomeratum* Thuill, Cyn: *Cynosurus cristatus* L., Dg: *Dactylis glomerata* L., Gr: *Geranium rotundifolium* L., Hl: *Holcus lanatus* L., J: *Juncus effusus* L., Lp: *Lolium perenne* L., Lt: *Lotus corniculatus* L., Or: *Ornithopus compressus* L., Pl: *Plantago lanceolata* L., Poa: *Poa pratensis* L., Pr: *Prunella vulgaris* L., Ra: *Rumex acetosa* L., Rll: *Rumex acetosella* L., Rn: *Ranunculus repens* L., Ro: *Rumex obtusifolius* L., Rp: *Raphanus raphanistrum* L., Se: *Senecio jacobaea* L., St: *Stellaria media* L. (Vill), So: *Sonchus oleraceus* L., Ta: *Taraxacum officinale* Weber, Tr: *Trifolium repens* L, Ue: *Ulex europaeus* L., Va: *Veronica agrestis* L

Understorey species richness was promoted by fertilization from 2001 to 2008. The exception was the treatment with 200 kg N ha^{-1} in the last year, probably due to the lack of the heterogeneity created by the tree, which covers the understorey by a 100%.

The dominant species after 8 years of planting was *Agrostis capillaris*, which is usually adapted to soils with low fertility (treatments 0 and 100 kg N ha^{-1}) and shade (200 kg N ha^{-1}). However, this species is not dominant in the treatment with 400 kg N ha^{-1} due to the smaller amount of shade than in 200 kg N ha^{-1} and the advantage that high fertility provides the high dose of sewage sludge which promotes a co-dominance with species better adapted to higher soil fertility, like *Holcus lanatus* and *Dactylis glomerata.*

Conclusion

Improving soil fertility increases grassland biodiversity if tree canopy cover is not complete.

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The effects of agricultural forages on soil biology – linking the plant-soil-invertebrate ecosystem

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Abstract

Soil biology is key to maintaining soil health, and soil health is fundamental to the sustainability of agricultural systems. Alternative forages have higher concentrations of essential nutrients, and different rooting patterns, potentially affecting soil-plant-animal interactions. Soil fauna have significant effects on belowground processes and are a vital part of carbon/nitrogen cycling, litter decomposition and the redistribution of nutrients. It is unknown how the soil food web will be affected by different forages, whilst all other environmental variables remain the same, under field conditions. An experiment was set up to test the hypothesis that alternative forages would alter the soil habitat leading to changes in soil biology. To investigate this, plots of chicory (*Cichorium intybus*), red clover (*Trifolium pratense*) white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*) were established in 2009. Plots were maintained over a three-year period, before soil biology samples were taken including soil mesofauna, nematodes, and earthworms. Significant differences were found between the forages and earthworm abundance, as well as some of the microarthropod groups. The implication of these results in relation to the soil food web and sustainable grassland systems is discussed.

Keywords: *Trifolium pratense*, *Trifolium repens*, *Cichorium intybus*, soil food webs, earthworms

Introduction

Agricultural profitability and sustainability are key to farming successfully, and with increasing feed and fertilizer costs, the use of home-grown alternative forages have increased. Advances in silage technology have allowed alternative forages to be ensiled as high-protein winter forage for livestock (Wilkins and Jones, 2000), giving farmers an option which may reduce their reliance on bought-in concentrate feed. Different forages have been shown to alter soil chemical composition due to their effects on the physical, chemical and biological properties of soil, due to differences in rooting depth (Culman *et al*., 2010) and N-fixation (Rasmussen *et al*., 2012). Agricultural grasslands usually support a relatively stable and numerous soil biota that contribute to soil functioning and fertility (Murray *et al*., 2012). Earthworms are often referred to as ecosystem engineers (Jones *et al*., 1994), because of their large effect over the soil environment (Blouin *et al*., 2013). Microarthropods also have significant effects on belowground processes and contribute to the carbon/nitrogen cycle and litter decomposition (Osler and Sommerkorn, 2007). To begin to understand the effect of different alternative forages on soil biology, an experiment was set up to test the hypothesis that alternative forages would alter the soil habitat leading to changes in the soil biology, in plots within the same field maintained under the same environmental conditions.

Materials and methods

The experimental area was set up at IBERS, Aberystwyth University (52° 25' 59" N 4° 1' 30" W) in 2009, and was uniformly ploughed to the same depth and standardized in accordance with RB209 guidelines (DEFRA, 2010). Field plots (7.5 m × 12 m) of five treatments were established in a randomized replicate block design (n=4). Perennial ryegrass

(*Lolium perene*) (cv. Premium) without any inorganic N ha^{-1} (0N), perennial ryegrass plus 200 kg N ha^{-1} (200N), chicory (*Cichorum intybus*) (cv. Puna II), white clover (*Trifolium repens*) (cv. AberDai) and red clover (*Trifolium pratense*) (cv. Merviot) were established at sowing rates of 33, 33, 6, 6 and 16 kg ha^{-1}, respectively. Between 2009 and 2012 the plots were mechanically harvested and the cut material removed; plots of ryegrass 200N and chicory had 200 kg N ha^{-1} annum^{-1}, applied as ammonium nitrate. No N fertilizer was applied to the plots of red clover, white clover or ryegrass 0N. Soil P and K indices were maintained at indices of 2+ using a dressing of 0-24-24 inorganic fertilizer applied at a rate of 60 kg P_2O_5 and 60 kg K_2O ha^{-1} annum^{-1}. In autumn 2012 and spring 2013, soil biological measurements were taken. Nematode abundance was assessed from a sub-sample of 200 g soil taken from across each plot to a depth of 15 cm, extractions were according to the methods of Whitehead and Hemming (1965) for wet tray extraction. Microarthropods were sampled from three intact soil cores collected from each replicate plot and bulked inside Tullgren funnels. Arthropods were extracted into 70% alcohol, over a 7-day period, prior to being counted and identification using a microscope (following Crotty *et al.* (2014)). Earthworm abundance and diversity was measured through the excavation of a 30 × 30 × 30 cm soil block from each plot, and through hand sorting to obtain the earthworms, before identification to species and biomass measured. All data were analysed using the statistical package GenStat (VSN International Ltd., Hemel Hempstead, UK) 14th edition. Data were analysed by a general analysis of variance (ANOVA) as a split plot, with treatment as the main factor. Where applicable, multiple comparisons were made using the Student Newman Keuls test.

Results and discussion

After the three years had elapsed and soil biology populations monitored, we found significant differences in earthworm abundance (Figure 1, *P* < 0.001), with the white clover treatment having greater abundances than all other treatments. Increases in earthworm abundance are known to increase organic matter consumption, soil structure modification and water infiltration (Blouin *et al.*, 2013), leading to greater nutrient mobility and improved soil health. We found no difference between nematode abundance between treatments (mean ± sem of 43 ± 2 total nematodes per gram dry soil). However, grouping the microarthropods into feeding guilds (*sensu* Crotty *et al.* (2014)) the two ryegrass treatments had significantly greater numbers of insect herbivores (*P* < 0.001), compared to the alternative forages, with ryegrass 200N having the greatest number (>5000 herbivorous microarthropods per m^2). The other microarthropod feeding guilds (microbivores, detritivores, omnivores, micropredators and macropredators) did not differ among treatments. Herbivores are known to reduce crop yields; therefore, in agriculture a reduction in numbers is preferable. Ploughing is known to reduce the heterogeneity of a field site (Hendrix *et al.*, 1986); doing this prior to the start of the experiment allowed the impact of the four different forages on soil biology to become more visible. We hypothesized that over the preceding three years after ploughing there would be a net movement of microarthropods (mesofauna, nematodes and earthworms) into or out of each of the treatments, depending on preference. Here, our results suggest the alternative forages, particularly white clover, are promoting a healthier soil environment in comparison to the ryegrasses. It is thought that the introduction of legumes has a key influence over how soil biota function, promoting soil structure, water retention, biodiversity and C and N storage (Murray *et al.*, 2012).
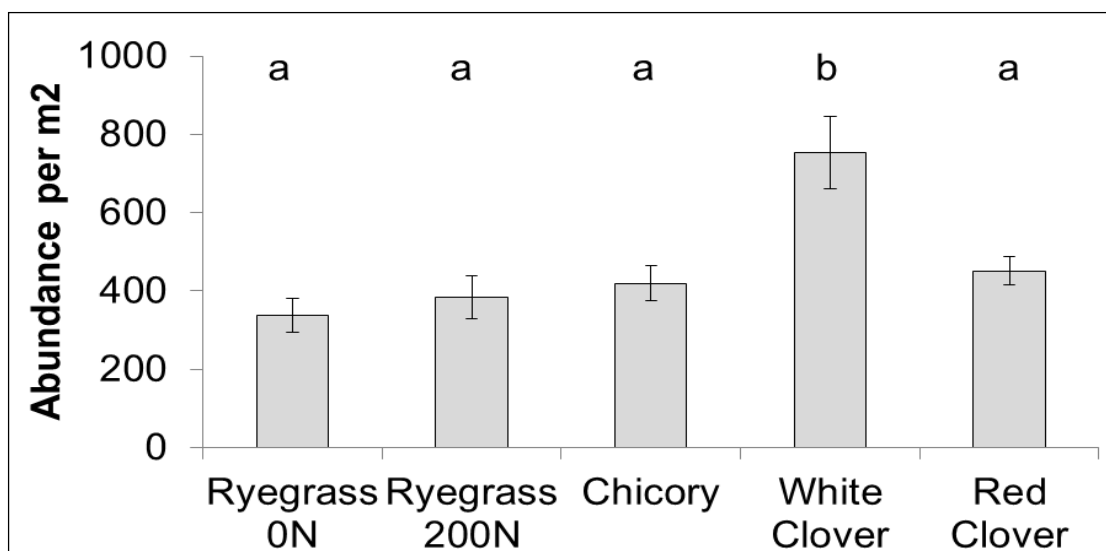
Figure 1. Mean total earthworm abundance (autumn and spring sampling period combined) per m^2 (±s.e; n=4). Significant treatment effects indicated by difference in letters ($P < 0.05$) analysed using ANOVA and SNK test.

Conclusion

Our study compares belowground soil food webs to assess the effect of growing different agricultural forage crops. Despite all plots being grown within close proximity to each other, within the same field, differences were found within the soil faunal groups. This suggests that over time there is an immigration / emigration from an area of soil dependent on crop type, and should be a consideration when implementing different sustainable farming methods.

Acknowledgements

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Managing grasslands to mitigate flooding risk

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Abstract

Grassland can be a significant source of surface runoff and can dominate flooding risk in some catchments. Mechanical loosening is often promoted as a means of improving soil structural condition and reducing surface runoff volumes. In 2010, four 'high' bulk density sites were selected to investigate the impact of shallower and deeper mechanical loosening, and interactions with the introduction of deep-rooting herbs and legumes. Double ring infiltrometers were used to measure both initial and saturated water infiltration rates in spring 2011, 2012 and 2013. Of all the treatments, deeper mechanical loosening had the greatest effect on both initial and saturated infiltration rates. Effects were greatest on the 'medium' soil sites, where deeper loosening resulted in saturated infiltration rates that were 4- to 10-fold greater than the un-loosened control ($P<0.001$). These results could have significant implications for the management of grassland soils in reducing surface runoff and flooding risk, particularly in catchments dominated by dairy and beef/sheep farms.

Keywords: Flooding risk, infiltration, soil structure

Introduction

In England, more than 5 million properties are at risk of flooding (nearly 1 in 6 homes). As a response to the devastating flooding in 2007, the Pitt Review provided a series of recommendations for improving the way flood risk is managed. The review advised that Catchment Flood Management Plans should be established to achieve greater working with natural processes such as using farmland to slow the progress of water to sub-catchment outlets and to minimize runoff. Grassland can be a significant source of runoff and sediment (Collins *et al.*, 2010) and there is a need to improve understanding of the implications of using mechanical loosening to increase water infiltration in grassland soils and potentially reduce peak flow in catchments that have a recognized flooding and erosion problem.

Materials and methods

In 2010, four 'high' bulk density sites were selected to test mechanical remediation of compaction and its interaction with the introduction of deep-rooting herbs and legumes (DHL) (Table 1). The following treatments were applied, using a split-plot randomized block design: i) an un-loosened and uncultivated control; ii) uncultivated with shallower (*c*. 20 cm) loosening; iii) uncultivated with deeper (*c*. 30-35 cm) loosening; iv) un-loosened and power harrowed without species introduction; v) power harrowed without species introduction with shallower (*c*. 20 cm) loosening; vi) power harrowed without species introduction with deeper (*c*. 30-35 cm) loosening; vii) power harrowed, DHL mix drilled and un-loosened; viii) power harrowed and DHL mix drilled with shallower (*c*. 20 cm) loosening; and ix) power harrowed and DHL mix drilled with deeper (*c*. 30-35 cm) loosening. There were four replicates of each treatment with individual plots measuring 8 × 16 m. Mechanical loosening was carried out using a grassland 'top-soiler' with leading discs followed by a set of tines and a single packer roller to the rear of the unit. Double-ring infiltrometers with an internal diameter of 600 mm (internal ring) were used to measure both initial and saturated water infiltration rates in spring 2011, 2012 and 2013. Water infiltration rates 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 treatments.

Results and discussion

Of all the treatments, deeper mechanical loosening had the greatest effect on both initial and saturated infiltration rates. Effects were greatest on the 'medium' soil sites (Nafferton, Odstone and Aberbran). At Bicton, the only 'sandy' soil site (topsoil clay content < 18%), deeper loosening increased what were already relatively high saturated infiltration rates in 2011, but there was no effect in subsequent years. By contrast, at the three 'medium' soil sites, mechanical loosening reduced infiltration rates into a third year, post-loosening. Mean saturated water infiltration rates in 2011, 2012 and 2013 are presented for all three 'medium soil' sites in Figure 1. Both shallower and deeper loosening resulted in higher (*P*<0.001) saturated infiltration rates (compared with the un-loosened control) in 2011 and 2012. By late winter 2012-13 (*c.* 30 months post loosening), infiltration rates were still higher (*P*<0.001) on the deeper loosened treatment compared with the un-loosened control. Notably, both initial and saturated infiltration rates in late winter 2012-13 on all three treatments were lower than in previous years, which may be a reflection of the very wet 2012 season.
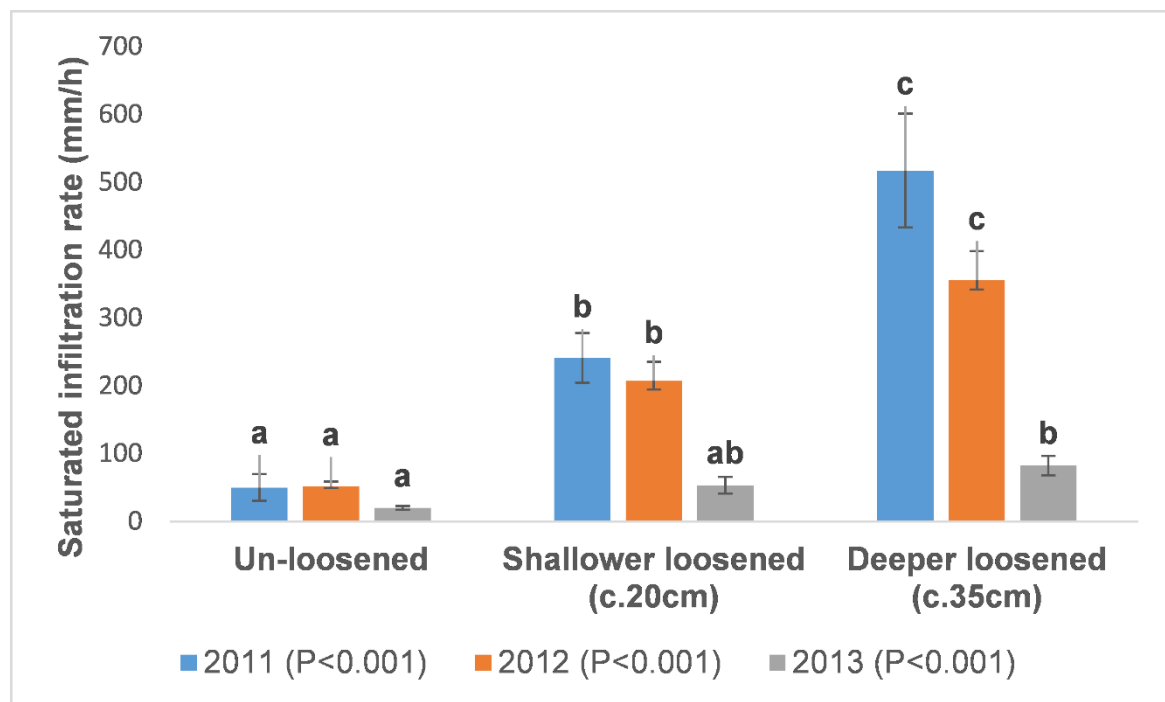
Figure 1. Effect of mechanical loosening on saturated water infiltration rates (mm/hour) on medium grassland soils with 'high' bulk density.

The saturated water infiltration rates on the unloosened plots compared favourably with 'typical' infiltration rates reported in MAFF Reference Book 441 (1982) of 150-500 mm/h for sandy soils (as at Bicton) and 30-50 mm/h for clay loam sites (Nafferton, Odstone and Aberbran). The above results, therefore, suggest that mechanical loosening (particularly deeper loosening) can increase water infiltration rates that are commonly found on managed grassland by 4- to 10-fold. This could have important implications for the management of grassland to increase slurry infiltration and for the reduction of flooding risk at the local scale. Within the time frame of this study, the seed mix had no effect on water infiltration rates. However, forbs and legumes can take a few years to establish so it is possible that rooting effects could be observed once plants have been well established for 4-5 years.

Table 1. Baseline soil analysis results for the four experimental sites.

Determinand	Units	Nafferton	Odstone	Aberbran	Bicton
pH	unit	5.9	6.9	5.6	5.7
Cross-compliance soil type	-	'Sandy and light silty'	'Medium'	'Medium'	'Medium'
Extractable Phosphorus (P)	mg/l	12	17	19	43
	[ADAS Index]	[1]	[2]	[2]	[3]
Extractable Potassium (K)	mg/l	91	210	76	125
	[ADAS Index]	[1]	[2+]	[1]	[2-]
Extractable Magnesium (Mg)	mg/l	170	145	77	68
	[ADAS Index]	[3]	[3]	[2]	[2]
Total Nitrogen (N)	% dm	0.29	0.25	0.21	0.17
Organic Carbon[1]	% dm	2.9	2.3	2.1	1.7
Organic Matter[2]	% dm	5.0	4.0	3.7	3.0
Bulk density	g/kg	1.36	1.44	1.27	1.30

Conclusion

Mechanical loosening can have significant effects on soil physical properties that persist for at least 30 months post-loosening. These changes in soil physical structure are associated with dramatic 4- to 10-fold increases in water infiltration rate that can also persist for a similar amount of time. Deeper loosening (to *c*. 30-35 cm depth) resulted in greater increases in water infiltration rate than shallower loosening (to *c*. 20 cm). Overall, it is concluded that deeper loosening is a more effective treatment than shallower loosening in improving grassland soil structural condition and function.

Acknowledgements

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Developing an *in situ* sensor for real time monitoring of soil nitrate concentration

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Abstract

Improving nitrogen-use efficiency is key to increasing the sustainability of livestock farming systems. Better management of nitrogen (N) inputs and waste resources is needed if significant improvements are to occur. However, farmers are currently limited by a lack of suitable, field-based tools for soil analysis and are overly reliant on limited, computer-based approaches. This project aims to develop an ion-selective electrode (ISE) capable of *in situ*, real-time soil monitoring of soil nitrate (NO_3^-). Construction of the electrodes in the laboratory is simple, low-cost and reproducible and the ISEs conform to theoretical norms. Current and future work will focus on the testing the performance of the electrodes in soil solutions and soils, and comparing the results to a range of standard methods.

Keywords: sustainable farming, nitrogen-use efficiency, ion-selective electrode, soil testing

Introduction

Optimizing the use of nitrogen (N) represents one of the major goals of sustainable livestock farming systems, from both an economic and environmental standpoint. While there have been thousands of studies investigating different management strategies for optimizing N use on farms, translating this research into practical management advice and subsequent adoption by farmers has often been unsuccessful. Consequently, as evidenced by numerous recent reports, there is no doubt that we have a long way to go before N is used efficiently within the UK livestock sector (Wilkins, 2008; Rees and Ball, 2010; Spiertz, 2010). The lack of significant improvement is partially due to a paucity of farmer-operated, field-based tools for soil analysis, and reliance on virtual (computer)-based approaches, which have limited adoption and lack precision (Cuttle and Jarvis, 2005). Ideally, adoption of simple field-based sensors that can monitor soil N in real-time will allow for more active management of fertilizer and waste resources, resulting in enhancement of fertilizer use efficiency, better timing of waste applications and reductions in environmental pollution.

Ion-selective electrodes (ISEs) have the potential to be used for real-time, *in situ* soil monitoring and have the advantages of being relatively inexpensive and easy to use (De Marco *et al.*, 2007; Sinfield *et al.*, 2010). However, current ISEs are not sufficiently robust enough for soil sensing and are subject to drifting calibration parameters, fouling and short lifespans (De Marco *et al.*, 2007). This project aims to develop a nitrate (NO_3^-) ISE which can overcome these challenges and bring about a step change in on-farm N management.

Materials and methods

ISEs function by measuring the potential difference between an electrode containing an ion-selective membrane and a reference electrode, which is not affected by the target ion. The activity of the target ion is related to the potential difference by the Nernst equation, which states that a ten-fold increase in activity of the target ion will result in a 59.1 mV change in electrode output. We construct the electrodes in our laboratory using a simple protocol and easily sourced materials. Briefly, both the NO_3^- and reference electrodes consist of a 1250 μl pipette tip, into which a PVC-based membrane is cast. The pipette tips are then back filled with

an unspecified solution, a Ag/AgCl wire is inserted, and the tip sealed. The electrodes are then coupled with a millivolt meter or suitable data logger.

Initial work has focused on characterizing the electrode's basic properties, including response time, the effect of interfering ions, developing a temperature compensation calculation and standardizing the manufacturing protocol to obtain a reproducible calibration (Buck and Lindner, 1994). Currently, we are testing the sensors in soil solutions and comparing the results to a standard colorimetric analytical method (Miranda *et al*., 2001) in order to assess the accuracy and precision of the sensors for NO_3^- determination. In addition, we are monitoring soil NO_3^- levels in the laboratory and assessing the performance of the sensors against a range of soil sampling techniques, including small tension lysimeters, centrifuge drainage and conventional soil core analysis. Following completion of this work, the sensors will be deployed in a grass-clover field trial in March–August 2014 to determine the differences in soil nitrate dynamics between different clover densities and inorganic N fertilizer amendments.

Results and discussion

Construction of the electrodes has proved succesful and reproducible, as seen in Figure 1.
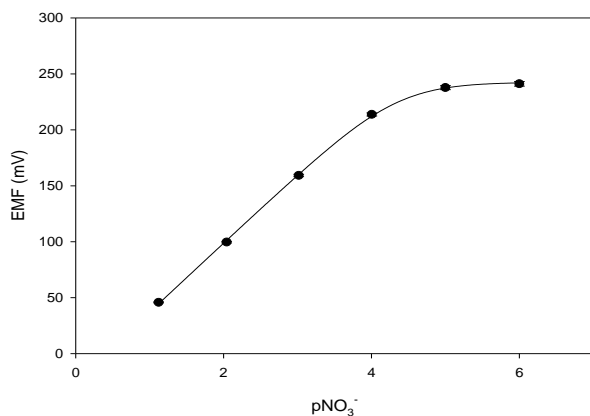
Figure 1. Calibration of NO_3^- ISE in standard NO_3^- solutions. Electromotive force (EMF) is the electrode output and pNO3- is the negative log of the NO_3^- activity. Data points represent means (*n=6*) ± *SEM* and the curve represents a modified Nernst equation.

These electrodes have a limit of detection of 47 µM and a near nernstian slope of 61.7 mV dec^{-1}.
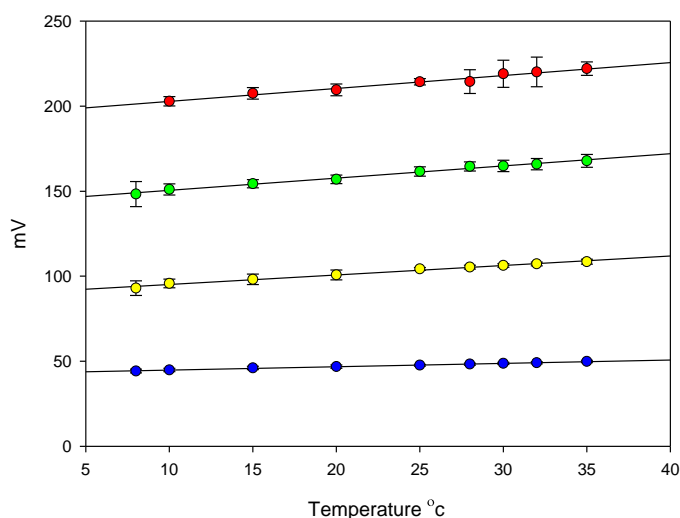
Figure 2. The effect of temperature on NO_3^- ISE output at different concentration of NO_3^-. Data points represent means (*n=6*) ± *SEM*

Figure 2 shows how electrode output (mV) is affected by temperature, with the magnitude of the change dependent upon the NO_3^- concentration of the sample. This is expected as the Nernst equation contains a temperature term, so the electrode outut can be easily adjusted using a simple calculation. Chloride is a well known interfering ion (Miller and Zhen, 1991) and the effect on these electrodes was determined using the fixed interference method (Buck and Lindner, 1994). The electrodes were recalibrated in the prescence of 100 mM Cl- and the selectivity coefficient was found to be 0.04.

Ongoing work and the results of the field trials will be reported on in September, as well as recommendations for industry as to how to take this promising technology further.

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Theme 2 posters

PastureBase Ireland – the measurement of grass dry matter production on grassland farms

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Abstract

Ireland in 2015 will produce milk without the restriction of milk quota. The future of an efficient low-cost milk production system will depend on low-cost feed in the form of grazed grass being converted to milk. On many farms some level of grassland measurement is being completed. To date, in Ireland there has been no central database to retain and archive these data for research purposes. The creation of PastureBase Ireland (PBI) in January 2013 addresses this issue. PastureBase Ireland is a web-based grassland database which has an inbuilt grassland decision support tool. The aim of the system is to build a large national grassland database which will increase the level of grass utilization and production at farm level. A key aspect of PBI is that the grassland farmer inputs the data at farm-level, through measuring farm grass covers regularly on the farm. For this paper, a subset of farms using PBI in 2013 was selected; all farms had a high level of grassland measurement to ensure the data used were accurate. The average DM production on the farms was 12.5 t ha^{-1}. There was significant variation in the amount of grass produced between farms, ranging from 16 t ha^{-1} to 8 t ha^{-1}. There was also significant variation between the average number of grazings per paddock achieved between farms, ranging from 9.1- 4.1. The implications of the grass growth data, the underlying reasons for variations in grass growth between farms and the variation that occurs within farms are discussed.

Keywords: PastureBase Ireland, grass, database, measurement

Introduction

The potential to produce between 12 and 16 t ha^{-1} grass dry matter (DM) over a long growing season is a major competitive advantage for Irish dairy farmers. Dillon *et al*. (2005) showed a strong relationship between total production costs and the grazed-grass proportion in the diet of the dairy cow in a number of countries. The average milk production cost was reduced by 1 c/L for a 2.5% increase in grazed grass in the dairy cow diet. The data also demonstrated that a considerable proportion of the dairy cow diet (>50%) must comprise grazed grass before a significant impact on production cost is realized. In recent years, grazing management strategies have been identified that increase the proportion of grazed grass in the dairy cow diet, which reduces the dependency on indoor feeding in Irish systems. The competitive advantage of grass-based production systems is expected to increase over the next few years due to higher costs associated with conserved and bought-in feed.

There is a requirement to refine and develop grassland technologies while also identifying the factors on-farm that are reducing grass growth. The development of a national grassland database which incorporates both a front-end, where farmers can enter grassland data, and a database, to collect all grassland data (commercial and research) would provide valuable strategic data for the entire grassland industry (dairy, beef and sheep enterprises).

With these goals in mind, Teagasc launched PastureBase Ireland (PBI) in January 2013. This was built from an in-house prototype. The database stores all grassland measurements within a common structure. PBI will allow the quantification of grass growth and DM production (total and seasonal) across different enterprises, grassland management systems, regions and soil types, using a common measurement protocol and methodology. The background data such as paddock soil fertility, grass/clover cultivar, aspect, altitude, reseeding history, soil type,

drainage characteristics and fertilizer applications are also recorded. All measurements are attached to individual paddocks within farms and PBI is used on both commercial and research farms. PastureBase Ireland will also, for the first time, link grass growth on farms to reliable Met Eireann weather data.

The objective of this paper is to examine grass growth data from a subset of farms in the PBI database and to identify the variations in grass growth that occur between farms and the variations that occur within a farm. The paper will also examine the economic loss of under-producing paddocks and strategies that farmers can use to increase grass growth on farms.

Materials and methods

Thirty-eight commercial grassland farms using the PBI decision support tool were selected to estimate grass production. Grass production was calculated from 1 January 2013 to 10 December 2013 (full grazing season). The farms used within this analysis were selected based on number of individual farm grass measurements, which had to exceed 30 grass measurements across the grazing season. All farms selected measured grass growth on each paddock weekly from January to December. The herbage mass on each paddock was measured either by visual assessment (calibrated by cutting and weighing) (O'Donovan *et al*., 2002) or by rising plate meter (Castle, 1976). Paddock growth was only calculated when the time between measurements did not exceed 16 days; all farms selected achieved this standard throughout the year. If farms did not have measurements within the 16-days window they were removed from the analysis. Both grazing yield and silage yield (estimated on harvest date) were calculated separately and then combined to give total grass production. The number of actual grazings achieved during the grazing season was calculated within the recording system. Average grass production (t ha^{-1}) was calculated across all farms and for different soil types. The data set was analysed in SAS (2003). PROC GLM was used to examine the effect of farm on total, grazing and silage DM production.

Results and discussion

Table 1 shows the grazing total DM yield, DM yield, silage DM yield and number of grazing's achieved on the farms for the entire 38 farms. Mean grass production across the farms (which ranged across Ireland) was 12.5 t ha^{-1}. The most productive farms produced up to 8 t ha^{-1} more than the lowest producing farms for both total and grazing DM yield. The number of actual grazing ranged from (9.1 - 4.1), which shows the level of grass utilization on the farms. The level of silage produced on some of these farms is low as farms are focusing on producing and utilizing grass as an in situ feed rather than as conserved forage.

Table 1. The total, grazing, silage DM production and number of grazings achieved on 38 grassland farms in 2013

	Total production (t ha^{-1})	Grazing production (t ha^{-1})	Silage production (t ha^{-1})	Number of grazings achieved
Mean	12.52	10.35	2.13	6.17
SED	0.48	0.56	0.45	0.329
CV	0.26	0.22	0.17	0.213
Sig	***	***	***	***

Farms which produced high levels of grass tended to have less between-paddock variations (this is measured by having a low CV – coefficient of variation) in comparison with farms producing lower levels of grass DM. Highly productive farms have adopted strategies such as regular monitoring of soil fertility, targeting reseeding on low-producing paddocks, reducing poaching damage, and continuing to maintain high levels of perennial ryegrass within paddocks to maximize production from individual paddocks. It is clear that there is huge economic loss

within and between farms due to variation in grass production. Shalloo (2009) found that increasing grass utilization was worth €160 per t DM utilized, and explained 0.44% of variation in net profit on commercial farms.

Conclusion

Grass DM production on the selected farms in 2013 averaged 12.5 t ha^{-1} which is considered a high level of grass growth. Large variation exists between the highest producing and lowest producing farms. The development of PBI both as a decision support tool and a grassland database is a hugely significant step for the future of grassland production systems in Ireland. PBI has the potential to add significant value to the data collected by individual farmers. These data show that there is significant potential for farmers to grow more grass on farms; however, the underlying reasons for poor grass DM production on individual farms and paddocks will need to be properly investigated and understood to achieve this potential. The creation of PBI will greatly assist this process.

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Estimation of grassland production with a new land classification system in Hungary

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Abstract

The future way of grassland management is greatly affected by the new functions of grasslands in relation to the environment. The evaluation of grasslands in Hungary is not solved: it is hard to plan the yields and we can estimate them only *a posteriori*. The D-e-Meter grassland module is a new grassland evaluation method. Our aim was the replacement of the disused classification system of grasslands, and the introduction of a new evaluation method as a function of sustainable agricultural developing. This aspect could be found in the multifunctional European Agricultural model and also in rural development. Therefore, we have carried out field experiments in the quest of solving this task in Bőszénfa, Southwest Hungary. The measured and estimated DM yield values were compared to each other for 5 grassland stands. The results showed that although there are significant differences between the estimated and the measured DM yields, the D-e-Meter method could provide a good basis to plan the management. The utility of the D-e-Meter method was confirmed by the evaluation of grassland plant products. These results certified the effects of different climatic features on grassland productivity.

Keywords: D-e-Meter, grassland, evaluation, yields, nutritive content

Introduction

Nowadays the usage of both arable and pasture land is controlled and has close connection to environmentally friendly farming. An important part of the possibilities of farm management is to know the actual quality and value of the fields (Várallyay, 2003). In the past, several scientists tried to construct methods for the estimation of the quality and value of grassland. Therefore, arable land evaluation and controlled farming are important issues. More classification systems of grasslands were applied in practice during the last decades, starting with Braun-Blanquet (1954), up to Ren *et al.* (2008). In Hungary, works of Máté (2003), Tóth *et al*. (2007) and Dér *et al*. (2008) are relevant. During the last ten years a new method, called D-e-Meter was carried out in Hungary, which hopefully will be suitable for the estimation of the characteristics of both arable land and grasslands (Tóth *et al.*, 2007). While this method will be suitable for arable farming, we have the aim to elaborate an up-to-date method for the grasslands, too. Classification of the grasslands must be solved in some ways. Baskay-Tóth (1966) separated grasslands into 3 groups according to their use: pasture, meadow and combined grasslands. Horn and Stefler (1990) classified grasslands by the intensity of their usage and separated them into three groups: intensive, semi-intensive and extensive pasture. A new way of grassland classification was created by Dér *et al.* (2003), which separates the grasslands by the way of their usage and the type of it: (1). Productive grassland (1a. unfertilized or barely fertilized grasslands with medium productivity; 1b. frequently fertilized grasslands with high productivity) and (2). Area of outstanding natural beauty (2a. strictly sheltered grasslands; 2b. non-strictly sheltered, and other grasslands of outstanding area; 2c. soil-protecting grasslands). Our aims were the formation of a more accurate system in the Hungarian grassland classification, replacing of the outdated 'Aranykorona-system' by means of this method, which imply both economic features and management facilities of grassland management.

Materials and methods

Our experiments were carried out from autumn of 2005, with soil analyses, until autumn of 2008, at the Game Farming Centre of the Kaposvár University, Bőszénfa. The examined five grasslands were: Baltacim (6 ha), Egyenestető (23 ha), Kuti III. (9 ha), Pacsirta (19 ha) and Templom Dél (20 ha). The meteorological details were measured by the Hungarian Meteorological Service. We measured three samples/cuttings per year from 2006 to 2008. The dates of the cuts are given in Table 1.

Table 1.Time of sampling (date, as year-month-days)

First growth (1)	Second growth (2)	Third growth (3)
2006.05.15–25.	2006.07.5–15.	2006.09.15–25.
2007.05.05–15.	2007.07.15–25.	2007.09.20–30.
2008.05.10–20.	2008.07.10–20.	2008.09.25–10.05.

The samples were cut in four repetitions using a quadrat frame and the weights of the samples were measured. The samples were analysed by dry matter (DM) contents, and the crude protein, crude fat, crude fibre, crude ash and N-free extract were quantified by Wendeei-analysis. The statistical analysis was done with SAS 9.1 software at 5% significance level *(P≤0.05)* by ANOVAs. D-e-Meter estimation method for grasslands is started from the potential DM values from the literature. The modifying factors were: quality factor, coverage factor, soil-water management, agro-ecological district, gradient category, sward establishment date, yearly climatic effect, intensification of farming and purpose of use (grazing or cutting). The complete methodology is compiled in Dér *et al.* (2008).

Results and discussion

The results of the measurements and the estimated values are compiled in Table 2. At 'Baltacim' we did not detect any significant difference between 2006 and 2007 in the fresh mass product per hectare. The DM yield per hectare and the crude protein yield per hectare also showed no significant differences between the experimental years. The drought of year 2007 had a great effect on arable land production, but grassland production was affected only in the second cut, so the other two cuts could equalize the yields. At 'Kuti III' we did not find any significant differences among the experimented yields. At 'Pacsirta' the annual change of the weather had a greater effect on the yields. In 2007 the unsuitable management had an effect too, so that the differences were significant in all the measured parameters. At 'Templom Dél' the differences were significant in the fresh mass yield in the experimental years. The levels of the yields were influenced by the weather and the land use management. Although there are significant differences between the estimated and the measured DM yields, the values show that the D-e-Meter method could give a good basis to plan the management. By improving this method by 0.2–0.4 t/hectare we can give estimations for the DM yield without significant differences.

Drought has the greatest effect on the botanical composition, the cover of the plants and the DM content in grasslands. In dry years the total number of the species can dramatically decrease, so this creates empty spaces, and both the number and mass of pioneer species (mainly weeds) increase. The DM content of the grasslands increased caused by the drought year of 2007, but the crude protein per DM kilograms and the crude fibre per DM kilograms were almost the same. The yields of the grasslands are influenced to a greater extent by the weather of the year (especially the rainfall) than are the nutritive contents. By evaluating the yields we can say that grasslands with greater yields have greater effect by the changes of the

weather than by the management. Grasslands can compensate for the environmental effects, which have impact on one cutting, compared to the field cropping species. The deviation between the estimated and measured DM yields is 0.05-12 t/ha, which shows a high level of deviation, but greater margin of error is in the droughty year, so we have to correct this point of the method. Of course, we must correct the accuracy of the method by further dates of the production, but all the results certify the conduciveness of the D-e-Meter system in the near future.

Table 2. Estimated and measured DM contents of the investigated grasslands, and the measured fresh mass and crude protein values.

Area name	Year	Estimated DM Yield (t/ha)	Measured DM Yield (t/ha)	Fresh mass (t/ha)	Crude protein yield (kg/ha)
Baltacim	2006	5.4[a]	5.4[a]	16.5[a]	631[a]
	2007	4.8[a]	5.6[b]	14.3[ab]	568[a]
	2008	4.8[a]	5.6[a]	13.8[b]	543[a]
Egyenestető	2006	6.7[a]	5.8[b]	20.2[a]	630[a]
	2007	6.0[a]	4.8[b]	14.3[b]	499[b]
	2008	7.5[a]	7.7[a]	23.2[c]	828[c]
Kuti III.	2006	4.5[a]	3.9[b]	13.0[a]	400[a]
	2007	3.0[a]	4.0[b]	12.1[a]	437[a]
	2008	4.5[a]	3.8[b]	11.7[a]	493[a]
Pacsirta	2006	6.7[a]	6.1[a]	22.7[a]	825[a]
	2007	2.0[a]	2.6[b]	8.2[b]	398[b]
	2008	6.7[a]	7.0[b]	23.1[a]	808[a]
Templom Dél	2006	7.1[a]	7.4[a]	23.6[a]	993[a]
	2007	4.8[a]	5.5[b]	15.1[b]	604[b]
	2008	5.4[a]	7.6[b]	17.6[c]	713[b]

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Influence of nitrogen fertilizers on yield and digestibility of grass

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Abstract

Forage in Latvia is mainly produced from grasses. Research was conducted with 5 grasses: reed canary grass (RCG) *var.* 'Bamse' (*Phalaris arundinacea* L.), tall fescue *var.* 'Fawn' (*Festuca arundinacea* Schreb.), festulolium 'Hykor' *(L. multiflorum x F. arundinacea)x FA,* timothy 'Jumis' (*Phleum pratense* L.), and meadow fescue 'Arita' (*Festuca pratensis* Huds.). Yield of dry matter in RCG biomass varied between 4.45 and 9.0 t ha^{-1}, in tall fescue from 4.3 to 8.16 t ha^{-1}, in festulolium from 2.5 to 7.36 t ha^{-1}, in timothy from 2.81 to 7.78 t ha^{-1}, and in meadow fescue from 2.79 to 7.36 t ha^{-1}. The results also show a close negative correlation (r = 0.8729, < 0.05), between content of crude fibre in the biomass and digestibility, and also between content of acid detergent fibre (ADF) and digestibility (r = -0.9990, $P < 0.05$).

Keywords: grasses, fertilizer norms, crude fibre, digestibility

Introduction

Sustainable livestock production systems are increasingly relying on production of high quality forage that can be produced on farm. Forage in Latvia, and other countries, is mainly produced from grasses. The value of plant green mass depends upon the digestibility of its organic matter; at the beginning of the vegetation period it comprises 80%, whereas at the end phase it falls to 50% or less. Plant digestibility is affected by lignin content, which links to ruminant crude fibre. Therefore, as plants mature, their digestibility of organic matter reduces. This phenomenon is clearly visible with the crude fibre (CF) fraction in respect to content of neutral detergent fibre (NDF) and the acid detergent fibre (ADF) content.

Changes of grass yield under the influence of various factors and climatic conditions of Latvia have been studied by several researchers (Adamovics, 1998; Runce, 1999; Bumane *et al.*, 2003; Gutmane *et al.*, 2008). Protein content in the grass dry matter is reported to be largely influenced by the plant development phase and nitrogen fertilizers applied (Adamovics, 1998). To produce high quality fodder and acquire high yields per unit area, research studied then influence of nitrogen mineral fertilizers on yield and digestibility of grasses.

Materials and methods

Field trials were carried out in 2011-2012 at the Research and Study farm "Peterlauki" (56° 53' N 23° 71' E) of the Latvia University of Agriculture, on sod calcareous soils, with pH$_{KCl}$ 6.7, available P of 52 mg kg^{-1}, K of 128 mg kg^{-1}, organic matter content 21-25 g kg^{-1}. Research was conducted with 5 grasses: reed canary grass (RCG) *var.* 'Bamse' (*Phalaris arundinacea* L.), tall fescue *var.* 'Fawn' (*Festuca arundinacea* Schreb.), festulolium 'Hykor' *(L. multiflorum x F. arundinacea)x FA,* timothy 'Jumis' (*Phleum pratense* L.), and meadow fescue 'Arita' (*Festuca pratensis* Huds.). Fertilizer levels used in the research (kg ha^{-1}) were: N$_0$P$_0$K$_0$ (control), P$_2$O$_5$–80 K$_2$O–120 (F– background), F+N$_{30}$, F+N$_{60}$, F+N$_{90}$, F+N$_{120\ (60+60)}$, F+N$_{150\ (75+75)}$, F+N$_{180\ (90+90)}$. Seed sowing was: 1000 germinable seeds per 1 m^2. The first cut pf the herbage dry matter (DM) yield was analysed in the Analytical Laboratory for Agronomy Research of Latvia University of Agriculture, for the following quality indices: crude fibre content (measured in compliance with LVS EN ISO 5498:1981), acid detergent fibre ADF (LVS EN ISO 13906: 2008) and digestibility – with cellulose method. The trial data were processed using correlation, regression and variance analyses (ANOVA) and descriptive

statistics with Microsoft Excel for Windows 2000 (Arhipova and Balina, 2006). The means are presented with their LSD test.

Results and discussion

Most of the DM yields were produced by the first cut of the grass. Average DM yield of the first cut in two years of production accounted for 62 -75% of the annual yield. Nitrogen fertilizers notably increased yield regardless the weather differences. The grass yield was influenced mainly by mineral-nitrogen fertilizers. The highest DM indicators were recorded for reed canary grass (9.06 t ha^{-1}), tall fescue (8.16 t ha^{-1}), timothy (7.78 t ha^{-1}) and meadow fescue (7.36 t ha^{-1}) with fertilizer level of F+N$_{180(90+90)}$, while that for festulolium (7.36 t ha^{-1}) was with F+N$_{120\,(60+60)}$ kg ha^{-1} (Figure1).
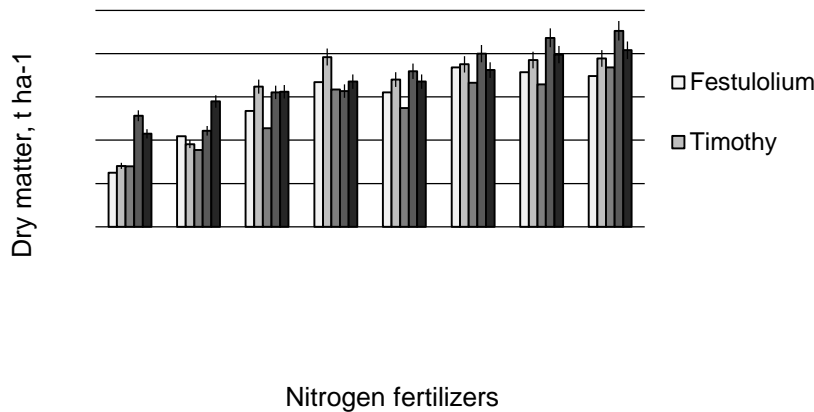
Figure 1. Dry matter yield of the first cut

The highest crude fibre content was found in meadow fescue (39.30 ±0.32%); in the biomass of other species it was notably (*P*<0.05) lower, the lowest being in timothy (35.72 ± 0.79%). Relation between crude fibre content in biomass and the digestibility was determined using correlation analysis (Figure 2).
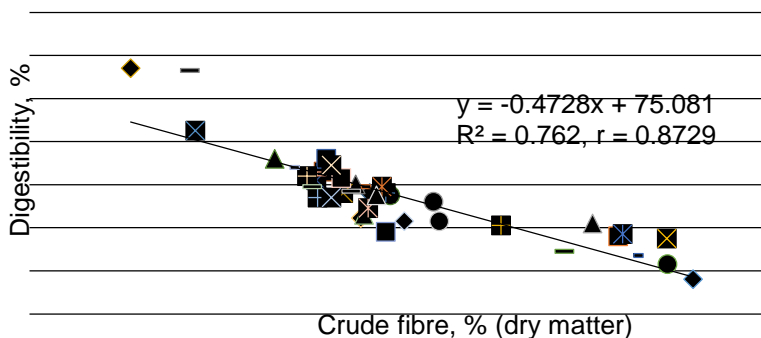
Figure 2. Results of digestibility and crude fibre content correlation analysis

The results obtained indicate close, negative correlation (r = 0.8729, *P*<0.05); thus digestibility reduces along with higher content of crude fibre in grass. Content of ADF has a significant effect on digestibility of fodder and on total energy value of the feed (Osītis, 2005). The highest ADF was recorded in festulolium (40.80 ±0.30%); in other species it was notably lower (*P*<0.05). The digestibility indicators varied notably among the grass species. The highest digestibility values were observed for timothy, tall fescue and festulolium (61.07 ± 0.62%, 60.01 ± 0.23% and 59.91 ± 0.11%, respectively), whereas the lowest was for meadow fescue

(57.11 ± 0.23%). Correlation analysis helped to find out the closeness of the relation between ADF content of biomass and digestibility (Figure 3.).

$$y = -0.786x + 89.158$$
$$R^2 = 0.9981, r = 0.9990$$
Acid detergent fibre, % (dry matter)
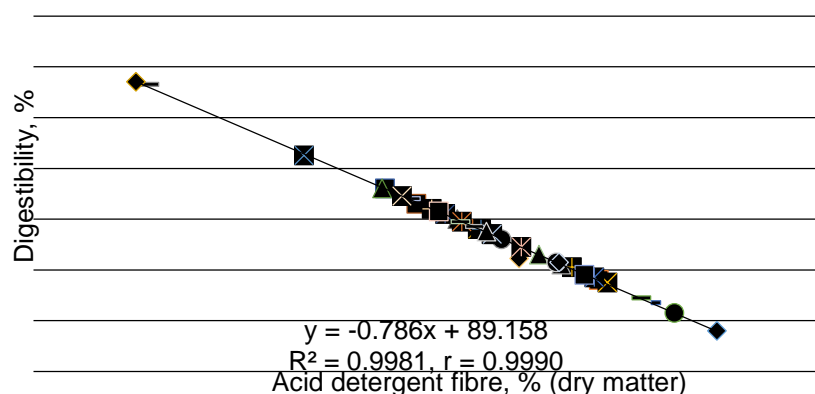
Figure 3. Results of digestibility and ADF content correlation analysis

Results of analysis show close, negative correlation (r = -0.9990, *P* < 0.05), thus as the ADF content in grass rises, the digestibility declines.

Conclusions

Nitrogen fertilizers increased the yield of dry matter. The highest DM yield in this research was found when grass received fertilizer at 180 kg N ha^{-1}. There is close, negative correlation between ADF content in the grass biomass and the digestibility; also crude fibre content in biomass and digestibility show close, negative correlation. Higher crude fibre and ADF content in the grass decreased the digestibility of forage.

Acknowledgements

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Forage yield and protein content of five native species from Lanzarote (Canary Islands)

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Abstract

The scarcity of fresh forage in the Canary Islands is a disadvantage for the development of cattle raising. Consequently, two-thirds of the food used for ruminants has to be imported. This dependence from the outside implies not only economic but also strategic problems for cattle exploitation. The objective of this work was to study the production and protein levels of five native valuable forage species from Lanzarote's Biosphere Reserve (*Atriplex halimus*, *Bituminaria bituminosa* var. *albomarginata*, *Coronilla viminalis*, *Echium decaisnei* and *Lotus lancerottensis*). This trial was developed in an experimental plot located on Lanzarote Island where three cuttings were carried out (winter and spring 2010 and winter 2011). The main forage production was obtained from *B. bituminosa* and the lowest from *C. viminalis*. On the other hand, the species with highest protein content were *A. halimus* and *C. viminalis* (166.4 g kg^{-1} and 153.2 g kg^{-1}, respectively) while *E. decaisnei* showed the lowest protein mean value (111.0 g kg^{-1}). The growth of these species could be of interest for dry zones.

Keywords: sustainability, cattle raising, Canary Islands, drylands

Introduction

The aridity and drought periods in Lanzarote Island (Canary Islands, Spain) cause the development of an active desertification process affecting 31% of its surface. The use of native species for the revegetation of the soil minimizes this effect and offers a possible solution, since these plants show a series of favourable characteristics. These species show quick growth, deep roots and high drought tolerance, while restoring and regenerating soils thereby preventing erosion (Chinea *et al*., 2004). Furthermore, their growth can be a source of cattle feed during periods of forage scarcity. The sustainability of this type of agriculture can represent an attractive option for farming production in territories with limited resources of quality water, as occurs in most part of the Canary Islands. According to 2010 Integral Cattle Plan data, Canary Islands is the region of Spain with the highest shortage in cattle feed production. The aim of this work was to study the production and protein levels of five native valuable forage species from Lanzarote's Biosphere Reserve (*Atriplex halimus*, *Bituminaria bituminosa* var. *albomarginata*, *Coronilla viminalis*, *Echium decaisnei* and *Lotus lancerottensis*), to offer a system for the harnessing of these species with interest for arid and semi-arid regions.

Materials and methods

Seeds from wild populations in Lanzarote Island were collected in July 2008 and germinated in forest containers (February 2009). Subsequently, they were planted (July 2009) in an experimental plot with 1387 m^2 located on the same island at 105 m above sea level (UTM X: 640.202; Y: 3.208.902). This plot showed a traditional mulching system and the soil comes from fertile plain-transported soils with clayish texture and alkaline pH (8.4), classified as Eutric Fluvisols. A random block design with four replications per species was established, employing sub-plots of 1.5 × 1.5 m. The plot was not fertilized during the experiment. Plants were irrigated with a dose of 1.33 mm/month/plant during the first year and 0.66 mm/month/plant the second year. The quality of water according to its electrical conductivity (EC = 0.54 mS cm^{-1}) implies no employment restriction, while the relationship between the

sodium adsorption rate (SAR = 3.2) and EC showed a light to moderate infiltration problem (Ayers and Westcot, 1985). During the period of the experiment, a mean temperature of 21.1ºC and a rainfall of 122 mm were registered. For all the studied species, cuttings were made when the main regrowths were greater than 30 cm. Three cuttings were made in total: January 2010 (winter 2010), June 2010 (spring 2010) and January 2011 (winter 2011). After cutting, the weight of all the green vegetal material was determined and 1 kg of each replicate was selected. Later, the edible fraction (leaves, green regrowths, flowers and non-lignified stems with diameters less than 5 mm) was manually selected and separated from the non-edible fraction. The edible fraction was dried at 60ºC for 48 hours and the production of edible dry matter (EDM) was determined. Samples were analysed for crude protein (CP) using the Kjeldahl distillation method. Data were statistically analysed using analysis of variance (ANOVA), and least significant difference (LSD) test was used for means comparison (SPSS statistics 17).

Results and discussion

The highest EDM production was obtained from *B. bituminosa* (2.11±0.42 t ha^{-1} and cutting), followed by *E. decaisnei* (1.69±0.25 t ha^{-1} and cutting) and *A. halimus* (1.65±0.19 t ha^{-1} and cutting). *Coronilla viminalis* (0.77±0.19 t ha^{-1} and cutting) showed the lowest mean production (Table 1).

Table 1. Mean values ± standard error of edible dry matter production (EDM) and crude protein (CP) per species. Species followed by different letters show significant differences ($P < 0.05$)

	Atriplex halimus	*Bituminaria bituminosa*	*Coronilla viminalis*	*Echium decaisnei*	*Lotus lancerottensis*
EDM (t ha^{-1})	1.65±0.19[bc]	2.11±0.42[a]	0.77±0.19[c]	1.69±0.25[ab]	1.42±0.25[abc]
CP (g kg^{-1})	166.4±6.8[a]	133.4±4.7[b]	153.2±11.2[ab]	111.0±8.9[c]	144.7±10.2[ab]

The production of *B. bituminosa* was notably lower than that mentioned by Méndez (2000) (19.2 and 12.9 t ha^{-1}), which reported a whole irrigation dose of 219 L m^{-2} and a high planting density (90000 plants ha^{-1}) compared with that one used in the present work (4444 plants ha^{-1}). Considering seasonal cuttings, the highest EDM production was obtained in spring 2010 followed by winter 2010 and winter 2011 (Table 2). Significant differences among all seasons can be observed ($P < 0.05$).

Table 2. Mean values ± standard error of edible dry matter production (EDM) and crude protein (CP) per season. Seasons followed by different letters show significant differences ($P < 0.05$).

	Winter 2010	Spring 2010	Winter 2011
EDM (t ha^{-1})	1.37±0.15[b]	2.14±0.28[a]	1.11±0.17[c]
CP (g kg^{-1})	164.8±6.4[a]	121.8±7.8[c]	141.8±5.9[b]

Atriplex halimus showed the highest CP levels (166.4 ± 6.8 g kg^{-1}), while the lowest appeared in *E. decaisnei* (111.0±8.9 g kg^{-1}) (Table 1). Significant differences were observed for the latter species compared with all the others. *Coronilla viminalis* (153.2±11.2 g kg^{-1}), *L. lancerottensis* (144.7±10.2 g kg^{-1}) and *B. bituminosa* (133.4±4.7 g kg^{-1}) presented intermediate levels and no significant differences were observed among them. Additionally, no significant differences were determined regarding the CP content for *C. viminalis*, *L. lancerottensis* and *A. halimus* (Table 1). Therefore, it could be highlighted that leguminous species (*C. viminalis* and *L. lancerottensis*) showed similar CP content to that reported by García-Criado *et al.* (1986) for the reference forage, alfalfa (148.0 g kg^{-1}). On the other hand, lower CP values were observed in *B. bituminosa* and *E. decaisnei,* while the highest was obtained in *A. halimus*. The highest CP concentration was obtained in winter 2010 followed by winter 2011, and the lowest mean values in spring 2010 (Table 2). Nitrogen content is higher when the growing conditions (temperature, light and water availability) are more favourable (McDonald *et al.*, 1988) and N

content decreases with plant age. This explains why in winter, with the highest vegetative regrowth rates in this Island (Chinea *et al*., 2013), the highest CP contents are reached.

Conclusions

The studied species showed high protein content and therefore could be of interest as forage crop in arid zones with low amounts of irrigation and without need of fertilization.

Acknowledgements

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Effect of manure enriched with clinoptilolite on pasture yield and quality

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Abstract

Since legislation imposes restrictions on the use of mineral nitrogen-based fertilizers, traditional organic fertilizers (such is cattle manure) are being applied and are now becoming a significant factor for achievement required forage production. Cattle manure offers available NH_4^+ for pastures; however, ammonia losses through leaching or evaporation significantly reduce its fertilization efficiency in crop production. Therefore, there is a need for a binding agent that can preserve nitrogen reserves. It is known from the literature that natural microporous alumosilicates – zeolites – can retain many different chemical species. Our previous results show that the addition of 10% by weight of zeolite tuff (70% by weight of clinoptilolite) to the fresh cattle manures binds 90% of the ammonia present in cattle manure. In the present study, cattle manure enriched with clinoptilolite was applied as a fertilizer. The experiments were carried out on natural pastures in 2012/13 and included four different treatments: pure fermented manure (30 t ha^{-1}); manure+zeolite (30 t ha^{-1}+10% zeolite); nitrogen-containing mineral fertilizer (50 kg ha^{-1} N) and control. The fertilizers were applied in December 2012, except mineral fertilizer, which was applied in spring 2013. The dry matter (DM) contents were estimated and the chemical analyses of forage done for two cuts. The implication for the future use of zeolites in sustainable grassland systems are discussed.

Keywords: clinoptilolite, manure, pasture, quality, yield

Introduction

Nitrogen (N) is crucially important as a yield-contributing element for most crop plants including forages. Restrictions in the use of mineral nitrogen in Europe (European Union Council Regulation No 834/2007) has changed the view on N fertilization of hill-mountainous grasslands of Serbia. Cattle manure as a fertilizer for pastures provides an available NH_4^+ source, but ammonia losses through leaching or evaporation significantly reduce efficiency of manure in crop production. Hence, there is a need for a binding agent that can preserve N reserves from manures. It is estimated that, for a period from excretion until collection to the farmyard manure (FYM) heap, that 40% of N is lost by evaporation during the warm half of year, while during a cold half of year 16% of N is lost (Moreira and Satter, 2006). After 6-months storage in the FYM heap, N comprised in FYM is assumed to be 80% of the collected N, due to gaseous losses (volatilization and denitrification; Petersen *et al.*, 1998). It has been shown previously that the addition of 10% (by weight) zeolite to fresh cattle manure increases retention of ammonia by 90% in comparison to the system without zeolite. Also, preliminary tests indicated that grass herbage yields obtained in a pot experiment containing ammonia-loaded zeolite were higher compared to control pots (Simić *et al.*, 2013). Kavoosia (2007) reported that clinoptilolite decreased the NH_4-N loss from the soil and enabled an easier uptake of nitrogen by the plant. The objective of this study was to investigate and launch the sustainable ammonia source for pastures in Serbia, which is based on natural zeolite-clinoptilolite mixed with manure, compared mutually and with mineral N supply. It is expected that the application of zeolite with manure can increase N use efficiency.

Materials and methods

Field plots (5m x 2m) were established on natural pastures in 2012/13 included five different treatments: a) pure manure (30 t ha^{-1}); b) manure+zeolite (30 t ha^{-1}+10 wt.% zeolite); c) pure zeolite (3 t ha^{-1}); d) nitrogen application by mineral fertilizer (50 kg ha^{-1} N) and e) control. The field trial was established in the vicinity of Šabac, Serbia, by the method of RCB design of plots in 4 replications. The zeolitic tuff (Zlatokop deposit, Vranjska Banja; containing 70% of clinoptilolite: $Ca_{1.6}Mg_{0.7}K_{0.7}Na_{0.3}Al_{5.5}Si_{26}O_{72} \cdot 23H_2O$, grain size in the range 0.063-0.1 mm) was used as the additive to manure in this work. Prior to application, fresh cattle manure was homogenously mixed with the natural zeolite – clinoptilolite, and fermented during 3 months. The treatments with zeolite, manure and a mixture zeolite+manure were applied in autumn. Spring nitrogen application of mineral fertilizer was performed at the beginning of the vegetation season. The plots were harvested in May and the dry matter (DM) of the harvests was measured. Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) were determined according to procedure by Van Soest; protein fractions (true protein and NPN) were determined as described by Licitra *et al*. (1996). The main characteristics of the soils were determined: pH in $CaCl_2$ was 5.07 while pH in H_2O was 5.73; the contents of P_2O_5 and K_2O were 19.8 mg kg^{-1} and 115.1 mg kg^{-1} respectively, total N was found to be 0.16%. Total DM yield and pasture DM quality in each of the two cuts were analysed by analysis of variance (ANOVA) and LSD test, in order to recognize significant effects of fertilization treatments.

Results and discussion

Fertilizer treatments affected yield, especially in the first cut: the yield was doubled by fertilization, in comparison with the control treatment (Table 1).

Table 1. Pasture dry matter yield and forage quality per cut. I treatment-control, II treatment- zeolite (3 t ha^{-1}), III treatment- manure (30 t ha^{-1}), IV treatment- manure+zeolite (mixed) (30 t ha^{-1}), V treatment- mineral nitrogen (50 kg ha^{-1}); DM – dry matter (t ha^{-1}), CP – crude proteins (% DM), CCl - crude cellulose (% DM), TP - true proteins (% of crude proteins), NPN-non protein nitrogen (% of crude proteins), NPN(%solP)-percentage of soluble protein that is non-protein nitrogen.

Treatments I cut	DM yield		CP	CCl	NDF	ADF	TP	NPN	NPN (%solP)
I	1.99c		9.71a	31.9a	62.8a	38.4a	73.6ab	26.4ab	89.4ab
II	2.38c		9.98a	33.0a	63.8a	37.8a	74.2ab	25.8ab	84.6ab
III	4.53a		10.1a	32.7a	63.9a	38.7a	64.8b	35.2a	100a
IV	4.11ab		9.86a	33.3a	63.4a	38.0a	83.5a	16.5b	65.7b
V	3.31b		10.5a	32.0a	62.3a	38.7a	69.6ab	30.4ab	96.2a
Treatments II cut	DM yield	Total yield	CP	CCl	NDF	ADF	TP	NPN	NPN (%solP)
I	1.02ab	3.01c	9.04a	25.9a	69.5b	37.1a	84.2ab	15.8ab	79.3a
II	0.91b	3.29c	9.02a	24.4a	66.6ab	41.3a	86.5ab	13.5ab	71.5a
III	1.38a	5.91a	9.72a	25.3a	66.7ab	38.8a	73.9b	26.1a	78.6a
IV	0.97ab	5.08ab	9.05a	25.0a	63.1a	37.9a	91.0a	9.0b	85.3a
V	0.96ab	4.27b	9.77a	27.3a	66.8ab	41.0a	80.7ab	19.3ab	93.8a

Manure showed some extended effect in the second cut, while the pure zeolite treatment had diminishing effects on yield in the arid conditions during summer regrowth. The most promising treatments are those with manure (enriched by zeolite or pure manure). Spring N application was also a good way to increase yield, whereas the pure zeolite and control treatments achieved the lowest yield. These results are in accordance with Gholamhoseini *et al*. (2013) who found that reducing of N leaching in the presence of natural zeolite, such as clinoptilolite, increases plant-available N and consequently increases N use efficiency. Also,

the present results obtained in Serbia confirm previously published data on natural zeolite combined with manure, which has been used successfully to remediate soils having unfavourable chemical properties as well as to enhance the crop yield on them (Glisic *et al*., 2009). It is very important to point out here that results obtained in this work indicate that the usage of zeolite-based fertilizer leads to an increase in the true protein content and decrease of non-protein nitrogen in grasses (Table 1), which can have a positive influence on forage digestibility. It may be concluded that the high true protein content of the plants grown on the plots with clinoptilolite can be caused by their ability to increase nitrogen uptake from soil due to the effect of clinoptilolite. It is in accordance with the conclusion of Gevrek *et al*. (2009) that the high protein content of the rice plants grown with clinoptilolite could be caused by their ability to increase nitrogen uptake.

Conclusion

The results obtained show that the use of cattle manure enriched with natural zeolite can be used as a fertilizer for pastures and this contributes to a preservation of nitrogen. The application of such a fertilizer may reduce the application of mineral N fertilizers on natural pastures. Based on the results presented here, natural zeolite can be recommended for agricultural purposes in terms of sustainable fertilizing and improving the system of cattle farm - manure - organic fertilizer for forage crops. Future studies should focus on including additional sites with different soil types in contrasting climatic areas.

Acknowledgement

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Influence of long-term organic and mineral fertilization on *Festuca rubra* L. grassland

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Abstract

Montane semi-natural grasslands are economically, ecologically and socially important, and therefore they require certain conservation measures. This paper summarizes the impact of organic, mineral and combined fertilization on the floristic composition of *Festuca rubra* L. grassland and includes results from an experimental field, which was established in the Apuseni Mountains (Romania). Fertilizer applications resulted in pronounced sward changes, depending on quantities applied. Even fertilization using low quantities changes the grassland type, so future conservation measures must consider this situation.

Keywords: semi-natural grasslands, organic fertilization, mineral fertilization

Introduction

Fertilization may generate major changes in swards and lead to a rapid reduction in species richness. Plant diversity is influenced by fertilizer type and amount, and by soil and climate (Vîntu *et al.*, 2011). Combining organic fertilization with mineral fertilization might be a good solution for maintaining the grasslands' species richness and also be associated with an appropriate increase the dry matter yield (Samuil *et al.*, 2012). Montane grasslands are important parts of the high natural-value domains, and in Europe they show great species richness. They could help increase local income from tourism and also be a seed source for restoring local biodiversity (Hopkins, 2011). *Festuca rubra* L. grasslands occur as fragmented areas in Central Europe, while in Carpathian Mountains they cover large areas. Their persistence considerably depends on their management. This paper's goal is to emphasize the impact of organic, mineral and combined fertilization upon the floristic composition in order to identify optimum conservation solutions for *Festuca rubra* L. grasslands.

Materials and methods

An experimental field was set up in 2001 in Gheţari village, Apuseni Mountains (Romania) at 1130 m elevation. The plots were arranged using the random blocks method with 4 variants in 5 replications (T_1 - control; T_2 - 10 t ha^{-1} manure; T_3 - 10 t ha^{-1} manure + 50N 25P 25K; T_4 - 100N 50P 50K; T_5 - 10 t ha^{-1} manure + 100N 50P 50K). The manure was provided by the cattle and horse husbandry. The inputs were applied each year in early spring. The floristic studies were performed according to the Braun-Blanquét method. The floristic data were processed by PC-ORD (McCune and Mereford, 2002). Vegetation ordination was done by Non-metric Multidimensional Scaling (NMS). NMS is generally the best ordination method for community data (Peck, 2010). The distance calculation was performed by Sorensen (Bray-Curtis) index. NMS was applied five times by the option Autopilot (Autopilotmode). In order to verify the final solution, the ordination was manually performed (6 axes, 250 runs, 250 iterations). The program recommended in both cases the two-dimensional representation to a stress in real data of 5.355 ($P<0.01$). Cumulative coefficient of determination for the correlations between ordination distances and distances in the original n-dimensional space was 0.909 (Axis 1 – 0.907, Axis 2 – 0.002). Also the MRPP (Multi–response Permutation Procedures) was used for testing the hypothesis of no differences between two or more groups of entities. The method implies the statistic test T which describes the separation between the groups. The *P* value is

useful for evaluating how likely it is that an observed difference is due to chance. The agreement statistic A describes within-group homogeneity, compared to the random expectation. A Monte Carlo test of significance is included.

Results and discussion

Festuca rubra L. plant community (control) is submitted to profound sward changes as a consequence of application of organic and mineral inputs application (Figure 1).
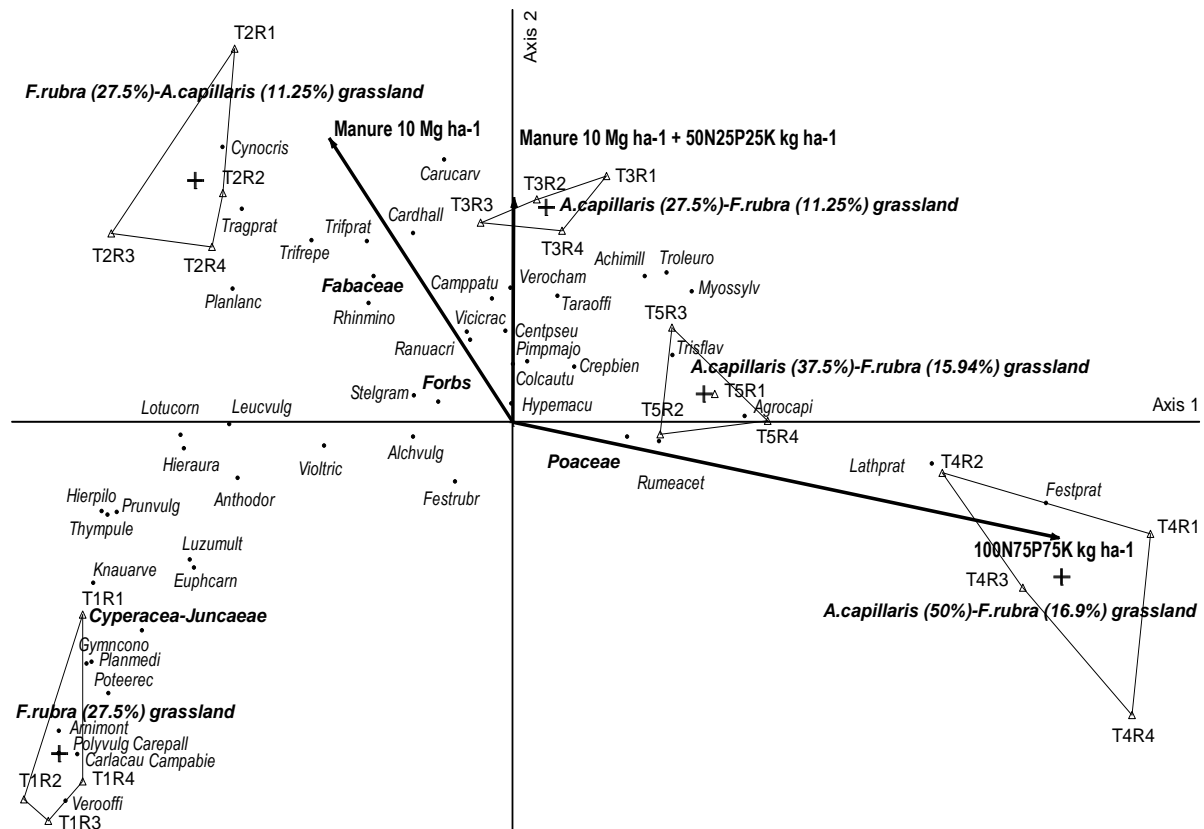
Figure 1. Influence of organic and mineral fertilization upon the floristic composition (T – treatments, R – repetitions)

Treatment performance generated the fixation of specific plant communities. When 10 t ha^{-1} manure were applied, besides the dominant species *Festuca rubra* L., *Agrostis capillaris* L. also occurred and became co-dominant (11.25%) and a large difference between T_1 and T_2 plant communities was registered *(P<0.01, Table 1).*

Table 1. The pairwise comparison between treatments with MRPP (T_1, T_2,…T_5– treatment,T- the t test, A - group homogeneity, P - statistical significance).

Comparison	T	A	P
T_1 vs. T_2	-4.10145197	0.41940328	0.00613211
T_1 vs.T_3	-4.28029428	0.59504554	0.00613211
T_1 vs. T_4	-4.39304453	0.64634213	0.00574951
T_1 vs. T_5	-4.33791511	0.63250901	0.00593859
T_2 vs. T_3	-4.11856121	0.33504342	0.00583843
T_2 vs. T_4	4.41742256	0.59348974	0.00565491
T_2 vs. T_5	-4.35817008	0.49167661	0.00567896
T_3 vs. T_4	-4.31111198	0.55182529	0.00593038
T_3 vs. T_5	-4.04164213	0.37275911	0.00650907
T_4 vs. T_5	-4.19750892	0.42446389	0.00606754

An increase in fertilizer quantity was marked by a greater share of *Agrostis capillaris* L. and a reduction of *Festuca rubra* L., which became co-dominant (Figure 1). When mineral fertilizers (T_4) were applied, the greatest proportion (50%) of *Agrostis capillaris* L. species was observed, whereas combining organic with mineral fertilizers (T_3, T_5) did not produced an extreme ratio between the co-dominant species. Among the plant communities generated by the three treatments (T_3, T_4, T_5), pronounced floristic differences were observed ($P<0.01$, Table 1), despite the fact that the plant community contained the same species. When performing a floristic study in the central part of Apuseni Mountains, Gârda (2010) all three plant communities were encountered, *Festuca rubra* L. showing the lowest share. Related to the economic groups of plants, it was noticed in the present experiment that grasses are favoured when large quantities of fertilizer are applied (T_4, T_5), legumes respond well to organic (T_2) and organic-mineral (T_3) fertilization, sedges and rushes did not prefer fertilization and forbs show the higher share by administrating T_2 and T_3 (Figure 1). T_2, T_3, T_4 treatments explain as statistically significant the floristic variation, whereas the particular effect of T_5 is difficult to distinguish from T_4 or T_3. In the control plant community there are numerous nitrophobic species which decline in proportion when other treatments were applied, or even disappear from the sward (*Arnica montana* L., *Veronica officinalis* L., *Carlina acaulis* L., *Campanula abietina* Griseb., *Gymnadenia conopsea* (L.) R. Br. *etc*.). Certain species prefer organic fertilization (T_2), such as: *Cynosurus cristatus* L., *Tragopogon pratensis* L., *Cardaminopsis halleri* (L.) Hayek., *etc*. Organic-mineral fertilization in moderate quantities (T_3) favours the following species: *Veronica chamaedrys* L., *Taraxacum officinale* Weber ex F.H.Wigg., *Trollius europeaeus* L., *Pimpinella major* (L.) Huds. *etc*. When large quantities of mineral fertilizers (T_4) and organic-mineral fertilizers (T_5) were applied, *Trisetum flavescens* (L.) Beauv*., Rumex acetosella* L. s. l., *Lathyrus pratensis* L. and *Festuca pratensis* L. species became established in the sward.

Conclusions

Dominance of *Festuca rubra* L. in grasslands is fragile. An annual application of 10 t ha^{-1} of organic manure does not maintain the control plant community with *Festuca rubra* L. as the dominant species. The evolution of *Festuca rubra* L. grassland brings a decline in the share, or even disappearance, of some oligotrophic species. In order to identify optimum possibilities to

preserve *Festuca rubra* L. grassland, studies that test other possibilities to apply organic and mineral fertilizers are necessary.

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Effects of low-input treatments on *Agrostis capillaris* L. - *Festuca rubra* L. grasslands

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Abstract

Traditional grassland management in the Apuseni Mountains has led to high biodiversity of the grassland ecosystems. The greatest risk for the future is the abandonment of these grasslands. In order to maintain and conserve these grassland systems the identification of new sustainable alternatives is required that area also economically sustainable. An experiment was installed in the Apuseni Mountains in 2009 to compare the effects of mowing, mulching, organic fertilization with mulching, and abandonment on oligotrophic grasslands. The highest number of species was influenced by organic fertilization combined with mulching. To maintain *Agrostis capillaris* L. - *Festuca rubra* L. phytocoenosis, the following treatments can be taken into account: organic fertilization combined with mulching, mowing, mulching.

Keywords: mulching, organic fertilizers, management, grassland, species composition

Introduction

Most of the species-rich semi-natural grasslands from different European regions, which are managed in traditional systems, are threatened either by intensification or abandonment. In the Apuseni Mountains (Romania) large areas of oligotrophic grasslands were maintained by applying an extensive and traditional management over a long period, where the most important links were organic fertilizers and mixed use (Gârda, 2010). The main economic resource (wood resources) of the region has suffered a drastic and continuous decline, which entails depopulation of the area. Despite the existence of subsidy programmes, areas of oligotrophic grasslands are abandoned. Thus, the identification of new, viable and low-cost alternatives is required in order to maintain and conserve these grassland ecosystems, combining traditional management elements with new ones for the region. Mulching could be an inexpensive alternative for the conservation of spices-rich semi-natural grasslands (Tonn and Briemle, 2010). The aim of the research was to follow the effects of mowing, mulching, organic fertilization combined with mulching, and abandonment, on plant-species composition of *Agrostis capillaris* L. - *Festuca rubra* L grassland and to identify new solutions for maintenance and conservation of these grassland ecosystems.

Materials and methods

The experiment started in 2009 in Poienile Ursului (1349 m), Ocoale Village, Gârda de Sus Commune, Apuseni Mountains, using a randomized block design with 7 treatments in 5 replications: T1 - control (mown 1 / year); T2 - mulch 1 / year; T3 - mulch 1 / year + 5 t ha^{-1} manure / year; T4 - mulch 1 / year + 5 t ha^{-1} manure / 2 years; T5 - mulch 1 / year + 10 t ha^{-1} manure / 2 years; T6 - mulch 1 / year + 10 t ha^{-1} manure / 3 years; T7 - abandonment. Cattle and horse manure was applied in early spring, according to the treatments. Floristic composition was interpreted by the Braun-Blanquet method and plots were then mulched once each year (August), the same time as the traditional mowing period. Floristic data processing was performed with PC-ORD version 6, which uses multivariate analysis of the data entered into the spreadsheet. This program focuses on nonparametric tools, on graphics, randomization tests, and bootstrapped confidence intervals for analysis of community data (McCune and Grace, 2011). For data processing and interpretation we used multidimensional scaling (NMS);

well suited to data coordination that are not normal, discontinuous, or otherwise questionable (Peck, 2010). This paper summarizes the results of the fifth experimental year, showing the effect of treatments on plant species composition of grassland. NMS was carried out several times in autopilot mode, in order to minimize the stress. Distance measurement was done with Sorensen (Bray - Curtis). The recommended solution for data presentation was tridimensional. The coefficient of determination *(r²)* for the correlations between ordination distances and distances in the original n-dimensional space was *0.740 (Axis1 - 0.421; Axis2 - 0.169; Axis3 - 0.151)*. NMS applied at plots level showed no significant results; for this reason we chose not to present these results within this paper.

Results and discussion

Changes in vegetation determined by different treatments, took place within the *Agrostis capillaris* L. - *Festuca rubra* L. phytocoenosis. Statistically significant experimental factors which explain the changes in floristic composition are: mowing, abandonment, mulching, organic fertilization combined with mulching (Fig.1a, Fig.1b). Axis 1 is correlated with organic fertilization combined with mulching *(r = -0.395)*, Axis 2 with mowing *(r = 0.317)* and abandonment *(r = - 0.317)* and Axis 3 with organic fertilization combined with mulching *(r = - 0.555)* and mulching *(r = 0.449)*. The gradual increase of organic fertilizers dose combined with mulching determines the increase in share of the following species: *Agrostis capillaris* L.*, Plantago lanceolata* L., *Rhinanthus minor* L., *Rumex acetosa* L., *Veronica chamaedrys* L., *etc*. Similar results have been obtained by Gaisler *et al.* (2004), where the species *Veronica chamaedrys* L., *Hypericum maculatum* Crantz, *etc*. were favoured by mulching once a year and *Agrostis capillaris* L.*, Plantago lanceolata* L., *Rumex acetosa* L., *Trifolium pratense* L. were favoured by mulching three times per year. *Veronica chamaedrys* L. presents a relative tolerance for shadow conditions and being able to resist also under a dense layer of litter (Pavlů *et al.*, 2003) and *Rhinanthus minor* L. is characteristic of species rich semi-natural grasslands with low productivity (Ellenberg *et al.*, 1991), being well known for taking advantage of hosts with high concentration in N (Ameloot *et al.*, 2008). The application of organic fertilizers combined with mulching led to a decrease in the percent of participation of the following species: *Arnica montana* L., *Centaurea mollis* Waldst. et Kit., *Festuca rubra* L., *Polygala vulgaris* L., *Silene nutans* L., *Thymus pulegioides* L. Mowing favours *Colchicum autumnale* L., *Euphrasia officinalis* L., *Ranunculus acris* L. and *Trollius europaeus* L. The abandonment determines the accentuated cover of *Hieracium aurantiacum* L., *Lotus corniculatus* L., and *Viola tricolor* L. Abandonment, even though it did not show a strong influence on vegetation, is not considered as a viable solution because it has been scientifically proven that in the long term it may produce profound changes. In our experiment mulching and/or fertilization combined with mulching favour *Trifolium repens* L. and *Trifolium pratense* L. (Fig. 1b). Effects of mulching, in particular at the species level, are very low. Mulching should be an acceptable solution, especially for already abandoned grasslands, because it could prevent the return of woody species (Gaisler *et al.*, 2011).
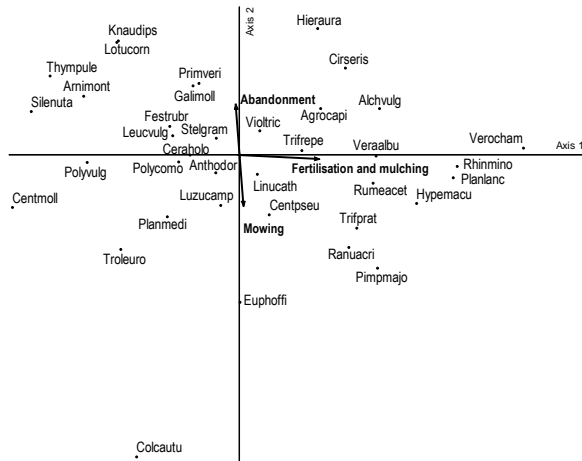
Figure 1a. Influence of the treatments on plant species composition (Axis 1 and Axis 2)
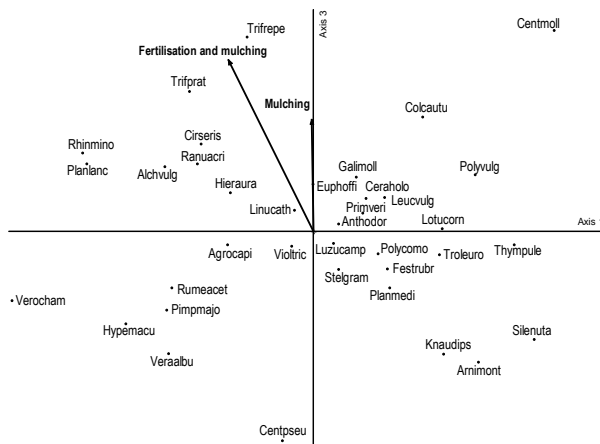
Figure 1b. Influence of the treatments on plant species composition (Axis 1 and Axis 3)

Agrocapi - *Agrostis capillaris* L., *Alchvulg - Alchemilla vulgaris* L.*;* Arnimont - *Arnica montana* L.; Centmoll - *Centaurea mollis* Waldst. et Kit.; Centpseu - *Centaurea pseudophrygia* C.A.Mey.; Ceraholo - *Cerastium holosteoides* Fr.; Cirseris - *Cirsium erisithales* (Jacq.) Scop.; Colcautu - *Colchicum autumnale* L.; Euphoffi - *Euphrasia officinalis* L.; Festrubr – *Festuca rubra* L., Galimoll - *Galium mollugo* L.; Hieraura - *Hieracium aurantiacum* L.; Hypemacu - *Hypericum maculatum* Crantz; Knaudips - *Knautia dipsacifolia* Kreutzer; Leucvulg - *Leucanthemum vulgare* Lam.; Linucath - *Linum catharticum* L.; Lotucorn - *Lotus corniculatus* L.; Pimpmajo - *Pimpinella major* (L.) Huds.; Planlanc - *Plantago lanceolata* L.; Planmedi - *Plantago media* L.; Polycomo - *Polygala comosa* Schkuhr; Polyvulg - *Polygala vulgaris* L.; Primveri - *Primula veris* L.; Ranuacri - *Ranunculus acris* L.; Rhinmino - *Rhinanthus minor* L.; Rumeacet - *Rumex acetosa* L.; Silenuta - *Silene nutans* L.; Stelgram - *Stellaria graminea* L.; Thympule - *Thymus pulegioides* L.; Trifprat - *Trifolium pratense* L.; Trifrepe - *Trifolium repens* L.; Troleuro - *Trollius europaeus* L.; Veraalbu - *Veratrum album* L., Verocham - *Veronica chamaedrys* L.; Violtric - *Viola tricolor* L.

Conclusion

After a study undertaken over a period of 5 years, the experimental factor that showed an influence on the greatest number of species was the organic fertilization combined with mulching, whereas mulching had the lowest influence. To maintain *Agrostis capillaris* L. - *Festuca rubra* L. phytocoenosis, the following treatments can be taken into account: organic fertilization combined with mulching, mowing and mulching. A particular attention should be paid to organic fertilization combined with mulching, because intensification of this factor could have an influence on high number of species.

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Influence of fertilization on the biodiversity of *Festuca rubra* L. and *Agrostis capillaris* L. grassland

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Abstract

The importance of semi-natural grasslands in Romania is shown by the area they occupy (4·9 million ha, which is 33% of the total agricultural area of the country) and their comparatively high biodiversity. In terms of area occupied by natural grasslands in Europe, Romania occupies fifth position after France, Britain, Spain and Germany. In Romania, meadows belonging to this category occupy an area of approximately 1,600,000 hectares and give relatively low production. The production of this semi-natural grassland is influenced by some natural and management factors. The objective of this paper was to evaluate the effects of fertilization upon the sward, in order to recommend certain versions which have minor repercussions upon the plant diversity. The experiment was set up in 2006 in mountain grassland of *Festuca rubra* L. and *Agrostis capillaris* L. In the experiment the effects of management treatments was evaluated on productivity and biodiversity of the grasslands. The use of a fertilizer management regime based on small quantities of organic nutrients can lead to larger yields while, at the same time, improving biodiversity.

Keywords: species richness, biodiversity, grassland, manure fertilization

Introduction

Semi-natural grasslands, traditionally used as forage for ruminants, are an important feature of land use in Europe, and cover more than a third of the European agricultural area (Pacurar *et al.,* 2012. Their fertilization with manure is considered an appropriate management to conserve biodiversity value (Peeters *et al.,* 2004). Several management factors may affect biodiversity of these grasslands including fertilization, overseeding, grazing and cutting management (Samuil *et al.,* 2012). In Romania, grasslands are an important forage resource, but irrational management systems during the last periuod have led to their present state of degradation (Vintu *et al.,* 2011). In this study, an experimental approach was used to evaluate the effects of management treatments on the biodiversity of *Festuca rubra* L. and *Agrostis capillaris* L. grassland. This paper presents the results of two experiments located at Pojorata, Suceava county, on natural grasslands of different floristic compositions.

Materials and methods

The experiment was performed on a meadow of *Agrostis capillaris* and *Festuca rubra*, at 707 m elevation on a slope of 20%, in Pojorata. The area has average temperatures of 6.3°C and 708 mm total annual precipitation. During April to September average temperature is 12.8°C with 514 mm rainfall. In terms of its climate, the area is situated to the north-east extremity of the European Central Province, with a temperate climate-moderate-continental, and some influence of the eastern continental climate and of the northern boreal climate. In the area where this research was conducted, the permanent grassland occupies an area of 156,000 ha, with a different distribution according to altitude, slope, temperature and precipitation.

The experiment was arranged in subdivided parcels, with four replications, and a plot size of 4 m x 5 m. Fertilization methods used and the amounts of nutrients supplied to the treatments were: A - *dose of manure*: $A_0 - 0$ t ha^{-1}, $A_1 - 10$ t ha^{-1}, $A_2 - 20$ t ha^{-1}, $A_3 - 30$ t ha^{-1}; B - *period of application of manure:* $b_0 -$ no manure, $b_1 -$ annually, $b_2 -$ every two years, $b_3 -$ every three years; C- *mineral nitrogen* which was applied every year at rates of: $c_1 - 30$ kg ha^{-1}, $c_2 - 50$ kg

ha^{-1}; and UAC - *apparent coefficient of use* (0·4 - for the manure applied annually; 0·45 - for the manure applied every two years; 0·55 - for the manure applied every three years). The chemical composition of 1000 kg of manure was 5.19 kg N, 2.83 kg P and 6.72 kg K. The manure was applied during the autumn, whereas the mineral nitrogen was applied in spring each year before vegetation growth began.

The grassland was managed by cutting with a Bertolini 411 harvester, and swards mown to a height of 4-5 cm above the ground. The harvesting was performed by mowing throughout the heading stage of the dominant grass. The final yield was expressed as dry matter (t DM ha^{-1}). Plant species composition analysis was performed using the number of species, the Shannon Wiener index, the Simpson index and the evenness index. In order to calculate these values, surveys were conducted at five points for each experimental parameter. We recorded presence/absence data and also the plant cover. The cover of all vascular plant species was visually estimated in each plot based on the Braun-Blanquet methodology. To eliminate edge effects, relevés were taken in the centre of each 4 m × 5 m plot within an area of 2 m × 3 m in mid-June 2013. The total number of vascular plant species was counted directly in the field (Cristea *et al.,* 2004).

Results and discussion

The botanical composition was very weak and was represented by species of low forage value (Table 1).

Table 1. Floristic composition and characterization of identified species (after Ellenberg *et al*., 1992).

Species	Indicators Ellenberg*					Species	Indicators Ellenberg*				
	L	T	W	R	Tr		L	T	W	R	Tr
Agrostis capillaris	7	x	x	x	3	*Trifolium repens*	8	x	x	x	7
Anthoxanthum odoratum	x	x	x	5	x	*Achillea millefolium*	8	x	4	x	5
Arrhenatherum elatius	8	6	5	7	7	*Alchemilla vulgaris*	6	4	6	x	6
Brachypodium pinnatum	6	5	4	7	4	*Carum carvi*	8	4	5	x	6
Briza media	8	x	x	x	3	*Leucanthemum vulgare*	7	x	4	x	3
Cynosurus cristatus	8	5	5	x	4	*Colchicum autumnale*	5	5	6	7	x
Dactylis glomerata	7	x	5	x	6	*Filipendula vulgaris*	8	7	4	x	3
Festuca pratensis	8	x	6	x	6	*Galium verum*	7	5	4	7	3
Festuca rubra	x	x	5	x	x	*Hypericum perforatum*	7	x	4	x	x
Holcus lanatus	7	6	6	x	5	*Knautia arvensis*	7	5	4	x	3
Nardus stricta	8	x	x	2	x	*Plantago lanceolata*	7	x	x	x	x
Poa pratensis	6	x	5	x	x	*Plantago major*	8	x	5	x	6
Trisetum flavescens	7	x	x	x	5	*Plantago media*	7	x	4	8	3
Anthylis vulneraria	8	x	4	8	3	*Prunella vulgaris*	7	x	x	4	x
Lotus corniculatus	7	x	4	7	4	*Ranunculus polyanthemos*	6	x	4	x	3
Medicago lupulina	7	5	4	8	x	*Rhinanthus rumelicus*	7	5	4	x	3
Trifolium campestre	8	6	4	6	3	*Rumex acetosa*	8	x	x	x	6
Trifolium montanum	7	x	3	8	2	*Taraxacum officinale*	7	x	5	x	8
Trifolium pratense	7	x	x	x	x	*Thymus pulegioides*	8	x	4	5	3

* Ecological indicators, according to Ellenberg *et al*., 1992: L = light value; T = temperature value; W = soil moisture value; R = soil (water) acidity (pH) value; Tr = trophic value

At the beginning of the experiment 38 species were identifies, of which 13 belonged to the family Poaceae, 7 to the Fabaceae, and 18 species belonged to other families. For these species some Ellenberg indicators were given. The following species were associated with the control: *Achillea millefolium, Agrostis capillaris, Briza media, Filipendula vulgaris, Nardus stricta, Plantago lanceolata* and *Potentilla erecta*. In contrast, *Arrhenatherum elatius, Dactylis glomerata, Festuca rubra, Lotus corniculatus, Trifolium pratense* and *Trifolium repens* were associated with the fertilizer N and manure treatments. The analysis of biodiversity parameters highlights (this was not tested statistically) that the number of species increased in all the variants where fertilizers were applied, compared to the control (Table 2).

Table 2. Diversity parameters determined in 2013

Variant of fertilization	Species richness (no.)	Shannon index	Shannon evenness	Simpson index (D)
A_0b_0	16	2.304	0.748	0.102
A_1b_1	20*	2.634*	0.823*	0.071*
A_2b_2	27**	2.742*	0.754 ns	0.082*
A_3b_3	18 ns	2.421 ns	0.814 ns	0.089 ns
$A_2b_1+A_1b_2+A_0b_0$	24*	2.534*	0.721 ns	0.092 ns
$A_2b_1+A_0b_0+A_1b_3$	21*	2.427 ns	0.826*	0.086*
$A_2b_1+A_1b_2+A_1b_3$	19 ns	2.441*	0.832*	0.091 ns
ns - non significant, *$P<0.05$, **$P<0.01$				

The number of species increased from 16, to 18-27 in the variants that were fertilized. Shannon index increased from 2.304 for the A_0b_0 treatment to values between 2.441 and 2.742 for fertilized treatments. Eveness Shannon was 0.748 at A_0b_0 and between 0.721 and 0.832 for the fertilized treatments. Simpson index was from 0.102 at A_0b_0 and was between 0.071 and 0.091 for the fertilized treatments. The increase of the number of species is attributed to the application of fertilizers. The manure was a source of increase in the number of species, especially in the category 'plants from other botanical families' because of the pool of seeds that it contains. The low doses of manure, applied at different intervals, together with low doses of chemical fertilizers, contribute to conserve the number of species.

Conclusions

The results showed that some changes occurred in plant species and functional groups of plants under the different fertilization treatments. The increase of the number of species is possibly due to improved soil nutrient supply on these lands and, in addition, to species being introduced with applied manure. Using a fertilization management based on small amounts of organic and mineral fertilizers can be a solution which will contribute to the conservation of the biodiversity of these grasslands. The results of this study, in an area considered to be regionally representative for large parts of the mountains of Romania, indicate that fertilization treatments are able to maintain a high diversity of species.

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The effect of organic fertilization on *Agrostis capillaris* L. and *Festuca rubra* L. grasslands from the Romanian Eastern Carpathians

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Abstract

Agrostis capillaris L. and *Festuca rubra* L. grasslands cover extended areas in Romania and represent a valuable source of fodder for bovines and sheep. The present paper presents the results of research conducted on a grassland from Bukovina, in the North of Suceava County, regarding the influence of manures in rates of 20-50 t ha^{-1} applied manually over the vegetal canopy, productivity and fodder quality. After three years of research there was an increase in the degree of vegetation cover from 87.3% to 99.9%, and an increase in the quantity of species from the *Fabaceae* - with increased vegetation cover from 3.5% to 37.8%. There was an improvement of fodder quality through the increase of CP content from 89.4 g kg^{-1} to 118.3 g kg^{-1}, a decrease of NDF, ADF and ADL content, and a rise of the RFQ from 73 in the unfertilized control to 90-99 in the fertilized variants.

Keywords: permanent grassland, organic fertilization, productivity, fodder quality

Introduction

The area covered with permanent grasslands in Romania amounts to over 4.8 m. ha, which represents 33% of the agricultural area. A large part of this area is situated in the mountainous regions, where grassland stands are the main food source for the animals. The increase in productivity and the improvement of fodder quality, with minimal effects for biodiversity and the environment, represent major objectives in the efficient use of these important natural resources (Cruz *et al*., 2002; Elsaesser *et al*., 2008). The quantity and the quality of livestock production depends largely on fodder quality, which in turn is influenced by the botanical composition, the vegetation stage at harvesting and the level of fertilization (Pozdisek *et al*., 2007; Rodrigues *et al*., 2007; Vintu *et al*., 2011).

Materials and methods

A monofactorial-type trial was established on *Agrostis capillaris* L. and *Festuca rubra* L. grassland in Putna, located in the North of Suceava County (47^{0}49' N, 25^{0}36' E; altitude 611 m asl). The trial consisted a randomized block design, with three replicates. The soil had 14.3 ppm P and 287 ppm K. Cpmposted and semi-composted manure, at rates of 20-30 t ha^{-1} and 40-50 t ha^{-1} respectively, were manually applied in early spring, and effects were monitored. Experimental variants were: V$_1$-unfertilized control, V$_2$-20 t ha^{-1}, V$_3$-30 t ha^{-1}, V$_4$-40 t ha^{-1}, V$_5$-50 t ha^{-1}. The composted manure had a content of 46 g kg^{-1} N, 18 g kg^{-1} P$_2$O$_5$, 51 g kg^{-1} K$_2$O, and the semi-composted manure had 33 g kg^{-1} N, 13 g kg^{-1} P$_2$O$_5$, 40 g kg^{-1} K$_2$O. The Kjeldahl method was used for the determination of the CP, and for NDF, ADF and ADL the Van Soest method was used, while RFQ was determined through the relation proposed by Undersander and Moore (2002). Determinations of fodder quality were carried out on samples from the first cycle of harvesting, the data representing the average for the years 2010-2012. The statistical interpretation of the results was conducted by an analysis of variance (ANOVA), calculating the least significant difference and the square correlations between the manure doses and the followed indicators.

Results and discussion

The obtained data reveal significant differences regarding the participation of species from various groups as far as the vegetation cover is concerned. Unlike the control variant a larger participation was identified, of 61.4%, for species from the *Poaceae* and of 22.4% for forbs, the fertilized variants revealing a significant increase in the species ratio from the *Fabaceae*, from 3.5% to 33.5-37.8%, with a superior fodder quality and positive effects on fodder quality (Table 1).

Table 1. Effect of organic fertilization on the degree of vegetation cover.

Species group	Vegetation cover (%)					*, **, *** - positive significant and °, °°, °°° - negative significant at the 0.05, 0.01, 0.001 probability level, ns=unsignificant
	V_1(Control)	V_2	V_3	V_4	V_5	
Grasses	61.4	53.2°°	56.3°	57.0ns	54.1°°	*P<0.05=4.4; P<0.01=6.4; P<0.001=9.6*
Legumes	3.5	37.8***	34.0***	33.5***	35.7***	*P<0.05=5.7; P<0.01=8.5; P<0.001=12.5*
Forbs	22.4	8.6°°°	9.6°°°	9.4°°°	10.1°°°	*P<0.05=2.5; P<0.01=3.4; P<0.001=5.4*
Total	87.3	99.6***	99.9***	99.9***	99.9***	*P<0.05=1.3; P<0.01=1.8; P<0.001=2.8*

The analysis of data from Table 2 reveals that all fertilized variants recorded a very significant progress in the production of dry matter, with a growth of 135-250% compared to the control variant (V_2, respectively V_5); this emphasizes the importance of manure in the increase of productivity for *Agrostis capillaris* and *Festuca rubra* grasslands in the Romanian Carpathians. As far as fodder quality is concerned, there was a positive influence of organic fertilization on CP content, increasing from 89.4 g kg^{-1} DM in the control V_1, to 118.3 g kg^{-1} DM in the V_5 variant, with statistically significant increased growth for the use of 30-50 t ha^{-1} manure rates.

Table 2. Influence of organic fertilization on the production of dry matter and the fodder quality.

Treatments	Dry matter (t·ha^{-1})	CP (kg ha^{-1})	CP (g kg^{-1})	NDF (g kg^{-1})	ADF (g kg^{-1})	ADL (g kg^{-1})	DDM (% DM)	TDN (% DM)	DMI (% BW)	RFQ
V_1 (control)	1.08	96.6	89.4	596.2	516.2	90.0	48.7	44.9	2.01	73
V_2	2.55***	254.2	99.7	556.8°°	455.2°	80.2°°°	53.4*	51.6**	2.14**	90*
V_3	3.38***	349.5*	103.4*	537.9°°°	453.5°	84.6°°	53.6**	51.8**	2.23***	94**
V_4	3.78***	407.4**	107.8*	517.3°°°	451.2°°	85.7°	53.8**	52.1**	2.33***	99**
V_5	3.80***	449.5**	118.3**	548.0°°°	441.3°°	86.9°	54.5**	53.2**	2.20**	95**
P<0.05	*0.46*	*190.1*	*13.8*	*21.9*	*44.4*	*3.1*	*3.3*	*4.0*	*0.09*	*13*
P<0.01	*0.66*	*282.3*	*20.1*	*31.8*	*64.6*	*4.6*	*4.8*	*5.8*	*0.13*	*19*
P<0.001	*0.99*	*421.2*	*30.2*	*47.8*	*96.9*	*7.2*	*7.2*	*8.7*	*0.20*	*29*

CP-crude protein, NDF-neutral detergent fibre, ADF-acid detergent fibre, ADL-acid detergent lignin, DDM-digestible dry matter, TDN-total digestible nutrients, DMI-dry matter intake, RFQ-relative forage quality

The increase in production and of fodder content in crude protein has led to quantities of 254.2-449.5 kg ha^{-1} CP, compared to just 96.6 kg ha^{-1} CP for the control, with statistically ensured growth for the manure doses of 30-50 t ha^{-1}. The fertilization also determined the reduction in NDF, ADF and ADL content for the fertilized variants compared to the unfertilized control, with statistically ensured differences. Thus the content of NDF decreased from 596.2 g kg^{-1} in DM in the sample variant, to 517.3 g kg^{-1} in DM in the V_4, and the ADF content decreased

from 516.2 g kg^{-1} in the control variant, to 441.3 g kg^{-1} in the V$_5$. The decrease of the NDF and ADF content has influenced the rise of the RFQ values in all the fertilized variants, with statistically ensured growth, the highest RFQ value of 99 being reached for the V$_4$. The RFQ growth compared to the unfertilized control varied between 23 and 34% (V$_2$, respectively V$_4$). Moreover, increases were observed in the DDM, DMI and TDN values compared to the sample one in all the fertilization variants, with statistically ensured growth, underlining the positive role of organic fertilization in fodder digestibility, following the reduction in content from the cell walls.

The analysis of the obtained data indicates that the regressions between the applied manure rates and the NDF and ADF content were negative, and with TDN values are positively correlated (Figure 1).
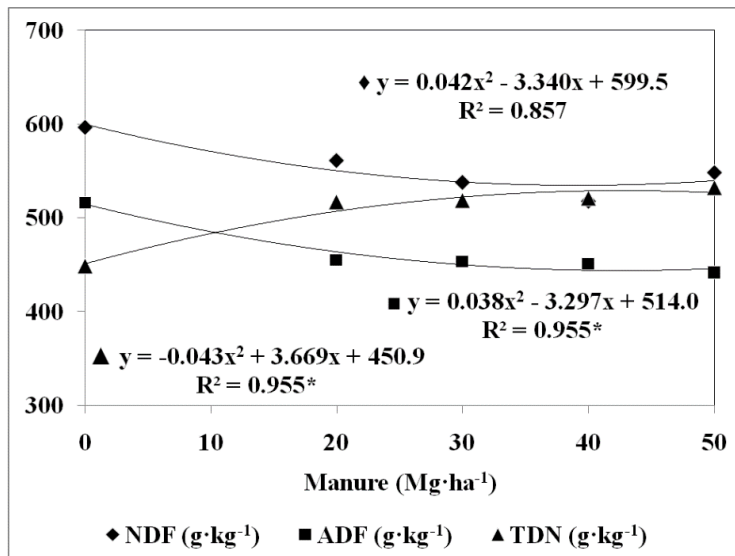
Figure 1. Correlations between the applied manure rates and the fodder content of NDF, ADF and TDN

Conclusions

A rational organic fertilization and its sustainable use has determined a change in the composition of floristic composition, an increase in the degree of vegetation cover, in productivity and the significant improvement in fodder quality, by reducing the content in the cell walls, increasing digestibilitty and the content of crude protein. The *Agrostis capillaris* L. and *Festuca rubra* L. grasslands from the Romanian Carpathians have the potential to become an important resource of fodder through fertilization with 30-50 t ha^{-1} of manure, thereby ensuring production growth of 212-250% compared with that of the unfertilized control.

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Herbage Recommended List applicability to low inorganic nitrogen (N) production systems

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Abstract

The Recommended Grass and Clover List (RGCL) trialling system is designed to evaluate varietal potential in which fertility issues are removed as a limiting factor. As plant nutrients are an essential part of plant development, with nitrogen (N) in particular being second only to photosynthesis in importance to potential yield, the key question is whether the expression of yield and quality traits under a high-input system are reflected in restricted nutrient supply systems. A series of field trials was established in 2011 across three United Kingdom locations. Six intermediate perennial ryegrass (IPRG) (*Lolium perenne.* L) varieties were selected with similar heading dates, and managed under a standard RGCL operating procedure while being tested under varying levels of N inputs (100, 200, 400 kg N ha^{-1}). Fertilizer regimes significantly ($P<0.001$ and $P<0.01$) affected IPRG yields and forage quality respectively. The highest levels of yield and quality were obtained from the 400 kg N ha^{-1} treatment. Yield performance rankings of IPRG varieties varied with management regime and individual cuts. There were no significant N-level varietal interactions; therefore, preliminary indications from this ongoing study are that the RGCL system represents the varietal performance irrespective of N fertilization regime.

Keywords: nitrogen, grass clover recommended list

Introduction

The current RGCL system for perennial ryegrass (*L. perenne*) assesses varieties' genotype x phenotype interactions of environmental and management factors (Burns *et al.,* 2013). The RGCL procedure utilizes high levels of inorganic fertilizer particularly N (c. 400 kg N ha^{-1}). This allows varieties to 'perform' without nutrient constraints and to achieve their true potential, and therefore identifies varietal differences. As a result the current RGCL trials represent a high-input system; however, grassland systems are diverse, from highly intensive to extensive semi-natural pastures (ADAS, 2009). Agri–environmental schemes such as the Entry Level Scheme (ELS) encompass 60% of England's farmed area. Within ELS there are options to reduce N inputs within areas of farmland (Natural England, 2013).

Grass species display differences in root growth, turnover rates and architecture Humphreys, 2011; Sokolovic *et al.,* 2013). Root morphology can significantly influence infiltration / percolation rates and N interception (Nichols and Crush 2007; Humphreys, 2011). Nichols and Crush (2007) demonstrated water percolation levels through the soil profile under hybrid (*Lolium x boucheanum*) and Italian ryegrass (*Lolium multiflorum* Lam) was half that of *L. perenne*, co-occurring with significant ($P<0.05$) differences in labelled ^{15}N interception. Studies in *L. perenne* have identified cultivar differences in both growth habit (Sokolovic *et al.,* 2013) and root morphology impacting on ^{15}N interception (Nichols and Crush, 2007).

A proportion of herbage cultivars within commercial grassland systems are not under high input systems. Cultivars also demonstrate differences in below-ground attributes. Therefore, varietal responses in terms of performance (yield/quality) under more restricted N supply may differ from the RGCL system. Preliminary work (NIAB, 2006) indicated some correlation between PRG variety performance under the current RGCL system and a low input system, and weak correlation under zero inorganic N. This study builds on the NIAB (2006)

preliminary work and focuses on the applicability of the RGCL system in the context of lower N production systems.

Materials and methods

Field trials were established in two sowing years in Yorkshire, Shropshire and Devon (on Aberford, Arrow and Teme soil series, respectively). The experimental design was a randomized block replicated four times and plot size was $6.5m^2$. It comprised six IPRG (*L. perenne*) diploid and tetraploid (T) varieties. Selected varieties were Premium (D), Rodrigo (D), Abergreen (D), Aubisque (T), Montova (T) and Seagoe (T). The varieties represent a relatively narrow heading-date range and a broader performance range in terms of yield and quality. Varieties were sown in accordance with their ploidy (25 ha^{-1} diploid, and 35 kg ha^{-1} tetraploid). Three annual fertilizer regimes (100, 200, 400 kg N ha^{-1}) were applied across the trial, with spring and post-cutting applications. Harvesting regime follows a system of successive harvest years incorporating conservation management (Year 1 and 3; circa 5 cuts/year) and grazing management (Year 2; circa 8 cuts/year) as per the RGCL protocol. Assessments included yield (t DM ha^{-1}) and quality (Digestibility value). All other fertility indices were maintained as per 'good agronomic practice' and designed not to be crop limiting. Data were analysed by analysis of variance and other appropriate statistical tests.

Results and discussion

N fertilization regimes significantly ($P<0.001$) influence grass yield under conservation and grazing management. The greatest yield was expressed under the high (400N) input system, consistent with established findings (St. Luce *et al.,* 2011). Analysis of quality of late-season digestibility value demonstrated significant ($P<0.01$) quality improvement with higher N levels. This is potentially attributable to physiological changes within the plants at differing N levels. Drought stress in the 2013 season and nutrient deficiency could have induced a higher proportion of stems and reproductive growth in the lower-N treatments. Increased stem production increases neutral detergent fibre (NDF) and acid detergent lignin (ADL) content and a higher ADL/NDF ratio, resulting in modifications to the digestibility value (Tas *et al*., 2005). Results are consistent with results reported by the Tas (2006), whereby phenotype N regime, management and cultivars genotype influenced herbage yield and composition.

The 2012 conservation-management annual yield demonstrated consistent ranking of varieties across the fertilizer regimes. Variety ranking within the 2013 grazing system (Table 1) displayed greater variability across the N-fertilization regime.

Table 1. Ranking of perennial ryegrass varieties under grazing management (2013: annual yield in t DM ha^{-1}). T denotes tetraploid varieties.

Rank	100 kg N ha^{-1}	200 kg N ha^{-1}	400 kg N ha^{-1}
1	Abergreen	Abergreen	Abergreen
2	Premium	Seagoe (T)	Montova (T)
3	Seagoe (T)	Rodrigo	Seagoe (T)
4	Aubisque (T)	Montova (T)	Premium
5	Rodrigo	Aubisque (T)	Aubisque (T)
6	Montova(T)	Premium	Rodrigo

The Meehan and Gilliland (2013) study indicates that minor temperature shifts significantly ($P<0.019$) impacted on *L. perenne* yields. The second sowing-year grazing management data will provide an insight as to the extent of the impact of temperature on varietal performance.

Ranking of cultivars fluctuated between N-fertilizer regimes and individual cuts, culminating in differences expressed within the annual grazing yields (Table 2) and between management regimes. The most pronounced ranking differences were expressed in the 2013 grazing-management system; however, there was no significant N x variety interaction with annual or individual-cut yields. This suggests that the performance of varieties (yield/quality) in terms of ranking is unilateral irrespective of fertilizer regime.

Conclusion

Nitrogen significantly influenced herbage yield and quality over the 2012 and 2013 season. Conservation management preliminary results under differing N fertilisation regimes appear to mimic RGCL ranking. Grazing management demonstrated the largest disparity from the RGCL ranking. Analysis demonstrates that no significant N regime x variety interactions, therefore the preliminary findings indicate the RGCL system represents lower fertiliser regimes. The second year sowings data and the 3rd year conservation management will provide a more robust data set to draw further conclusions.

Acknowledgement

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Effect of mineral fertilization on yield and quality of grassland ecosystem *Agrostietum vulgaris*

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Abstract

The main objective was to examine the influence of mineral fertilization on the production capacity of *Agrostietum vulgaris*-type meadow located in western Serbia, during 2005-2008. Mineral fertilizers with different NPK rates (0-500 kg ha^{-1}) were applied. Results established that mineral fertilization at $N_{200}P_{150}K_{150}$ provided the highest yield of dry matter (8.13 t ha^{-1}). On unfertilized soils dry matter yields of the grass sward was substantially lower and disappearance of valuable grasses was observed. As opposed to the unfertilized plots, on plots fertilized with high NPK rates herbs and weeds diappeared. We examined the effects of fertilizer application on hay and protein yield to avoid economic losses from loss of applied fertilizers.

Keywords: fertilization, meadow, DM yield, forage quality

Introduction

Natural meadows cover large areas in the hilly-mountain region of Serbia. They are of considerable importance for forage and soil utilization and protection. Because of poor management and careless utilization these grasslands are now degraded, with low production and poor quality. The use of fertilizer is an important factor in intensive grass-based dairy farming, as NPK affects dry matter yield and crude protein content of herbage. Efficient fertilization provides plants with nutrients at appropriate proportions and quantities which thereby enable maximum yield increase of crops with high biological and technological quality (Barabasz *et al*., 2002).

One of the highest presented and economically important associations in Balkan peninsula, at least in hilly-mountainous regions, is *Agrostietum vulgaris* (Tomić *et al*., 2009a). The association *Agrostietum vulgaris* in Serbia could include 47 plant species, of which 11 (or 23.4%) are useful grasses, 15 (or 32%) are useful legumes, 3 or (6.4%) are useful; however, less useful, are 17 (or 36.17%) that are are bad and worthless, with only one or (2.13%) harmful, but no poisonous species. *Agrostietum vulgaris* has a realized production of herbage mass of 3.15 t ha^{-1}, and of dry matter 1.10 t ha^{-1}. Content of crude protein was 10.0%, crude lipids 2.3%, crude fibre 29.8%, and NFE 41.7%. It realized the lowest production with regard to green mass and dry matter. The objective of this study was to assess the effect of fertilization on the yield and quality of semi-natural meadow type *Agrostietum vulgaris*, with respect to agroecological and economical conditions.

Materials and methods

Examination was carried out during four years (2005-2008), on a semi-natural meadow dominated by *Agrostietum vulgare* in the western region of Serbia (near Valjevo city). The experiment was a randomized block design with four replications. It included six fertilizer rates (0, 150, 200, 300, 350 and 500 kg ha^{-1} NPK yr^{-1}), which were applied in early April. The data determined in this experiment are: dry matter yield (DM), crude protein (CP), crude fibre (CF) and nitrogen free extracts (NFE) contents. Data were analysed as a factorial design by ANOVA; differences between means were determined by LSD.

Results and discussion

Effect of fertilizers on meadow-pasture vegetation is complex. The grass cover responds not only in terms of yield and quality but also in plant composition. Continued use of fertilizers changes the botanical composition of plant association. At the study site, *Agrostis vulgaris, Festuca rubra, Dactylis glomerata, Festuca pratensis and Arrhenatherum elatius* made the most important contribution to herbage yield in all the treatments.

The rainfall over the four growing seasons varied between 755 mm and 930 mm (Table 1). In the first and third year, rainfall was 6 and 8% less than the long-term annual rainfall of 820 mm for the study area, as opposed to the second and last year with 13% and 0.5% on average more than the long-term average.

Table 1. Dry matter production (t DM ha^{-1}) nutrient use efficiency (NUE) and seasonal rainfall use efficiency (RUE) (kg CP ha^{-1}mm^{-1}) on *Agrostietum vulgaris* grassland at different fertilizer application rates of N, P and K for the 2005-2008 growing seasons. Least significant differences (LSD) are calculated at the 5% level

Treatments	2005			2006			2007			2008		
kg ha^{-1}	DM	NUE	RUE	DM	NUE	RUE	DM	NUE	RUE	DM	NUE	RUE
$N_0P_0K_0$	2.32	0	3.01	2.15	0	2.32	1.47	0	1.94	1.89	0	2.3
$N_{50}P_{50}K_{50}$	5.20	19.2	6.75	4.52	15.78	4.86	3.20	11.5	4.24	4.59	18	5.57
$N_{100}P_{50}K_{50}$	6.22	19.5	8.07	5.8	18.25	6.24	4.35	14.4	5.77	6.62	23.6	8.03
$N_{100}P_{100}K_{100}$	6.25	13.1	8.1	5.85	12.33	6.3	4.7	10.8	6.23	6.39	15	7.76
$N_{150}P_{100}K_{100}$	7.01	13.4	9.1	7.9	16.41	8.5	5.73	12.2	7.59	6.62	13.5	8.03
$N_{200}P_{150}K_{150}$	8.27	12	10.73	8.11	11.91	8.72	7.11	11.3	9.41	9.03	14.3	10.96
LSD	0.75			1.37			0.83			1.95		
Average	5.88			5.72			4.43			5.86		
Precipitation		771			930			755			824	

Increased fertilization led to a higher (*P*<0.05) production over the four seasons (Table 2).

Table 2. Crude protein (CP), crude fibre (CF), ash and nitrogen free extracts (NFE) in *Agrostietum vulgaris* DM depending on fertilization (%) during the four-year period, 2005-2008.

Main Effect	CP	CF	Ash	NFE
Fertilizer treatment				
$N_0P_0K_0$	8.89	30.72	1.63	48.39
$N_{50}P_{50}K_{50}$	8.55	32.79	1.49	47.26
$N_{100}P_{50}K_{50}$	8.75	33.83	1.31	46.81
$N_{100}P_{100}K_{100}$	9.34	34.38	1.63	44.32
$N_{150}P_{100}K_{100}$	10.93	33.45	1.10	45.41
$N_{200}P_{150}K_{150}$	10.81	32.22	1.79	45.47
LSD 0.05	*1.42*	*2.23*	*0.55*	*3.1*
Years				
2005	11.74	32.71	1.74	47.80
2006	9.15	37.24	1.79	45.76
2007	9.28	29.73	1.17	45.68
2008	8.02	31.92	1.27	45.88
LSD 0.05	*1.16*	*1.82*	*0.45*	*2.53*

Compared with other seasons, the high rainfall during the 2005/06 season was insufficiently converted into dry material. This may be due to the poor seasonal distribution. The increase in production with equal $N_{50}P_{50}K_{50}$ application rates differed significantly (*P*<0.05) from that of the control, but increase was more significant with additional N spring rates. With NPK fertilization the

DM production peaked in the third treatment ($N_{100}P_{50}K_{50}$ with average NUE=19) in despite of higher NPK rates on the following treatments. This leads to the conclusion that the equal quantities of NPK were insufficient to ensure a rapid reaction in production or it may have a longer residual effect. Mean herbage yield increased from 1.96 t ha^{-1} DM (control plot) to 8.13 t ha^{-1} year^{-1}.

Figure 1 shows crude protein (CP) production (kg CP ha^{-1}) and seasonal rainfall use-efficiency (RUE) (kg CP ha^{-1} mm^{-1}) of *Agrostietum vulgaris* grassland at different NP fertilization rates and for the four growing seasons. In treatments N_{60} and N_{120}, the CP content in grass DM increased by 0.14 and 2.66%, and CP yield per hectare by 98 and 226%, respectively, compared with N_0 (at P_{60} K_{80} background). Other tests performed in Serbia showed that fertilizer N had a favourable effect on the yield, protein, ash and fat content while decreasing cellulose content (Vuckovic *et al.*, 2005a).
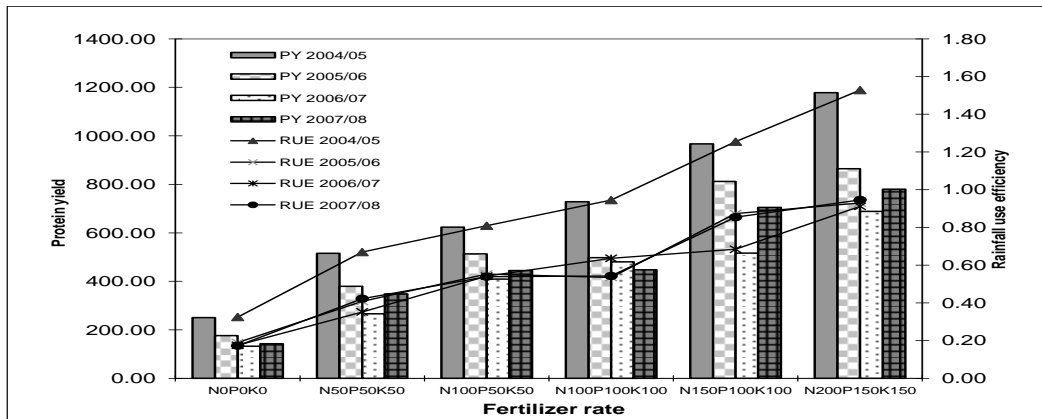
Figure 1. Crude protein production (kg CP ha^{-1}) and seasonal rainfall use-efficiency (RUE) (kg CP ha^{-1} mm^{-1}) on the *Agrostietum vulgaris* grassland at different fertilizer rates of N, P and for four growing seasons.

Conclusion

The data obtained in this study indicate that fertilization has a considerable influence on semi-natural meadows dominated by *Agrostietum vulgare*, regarding their DM yield and quality as well as botanical composition. The maximum four-year average DM yield of 8.13 t ha^{-1} and CP yield of 877.5 t ha^{-1} was achieved with the $N_{200}P_{150}K_{150}$ rate (500 kg ha^{-1} year^{-1}), but nutrient use efficiency was greatest with the $N_{100}P_{50}K_{50}$. The increased application of NPK fertilizers improved seasonal rainfall use efficiency and affected crude protein and fibre content.

Acknowledgements

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Impact of surface fertilization on dehydrogenase activity in grassland soil

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Abstract

Soil microorganisms have a significant role in soil processes and their activity is largely influenced by fertilization. The aim of this study was to determine the effect of fertilization on grassland soil dehydrogenase activity, which is one of the most sensitive bioindicators connected to soil fertility and microbial activity. Soil samples were collected after fertilization with mineral fertilizer, cattle slurry and cattle slurry digestate throughout the growing period in 2013 from grassland. Dehydrogenase activity was determined colorimetrically after sample incubation with iodonitrotetrazolium chloride. We found that the impact of surface fertilization on dehydrogenase activity was low. The dehydrogenase activity depended on air temperature more than on fertilization

Keywords: dehydrogenase activity, fertilizer, grassland, temperature

Introduction

Soil microbiological activity plays a key role in nutrient cycling. Its activity is essential in both the mineralization and transformation of organic matter and plant nutrients in soil (Dick and Tabatabai, 1993). Organic and inorganic fertilizers increase nutrient availability to plants, but at the same time they can affect the population, composition, and function of soil microorganisms (Marschner *et al*., 2003) and consequently soil enzymatic activities (Wolińska and Stępniewska, 2012). It has been found that organic fertilizers increase soil enzyme activities, while inorganic fertilizers have relatively less effect (Mijangos *et al*., 2006; Chu *et al*., 2007), but also that dehydrogenase activity increases by mineral fertilizer use (Chu *et al*., 2005).

Dehydrogenase activity (DHA) is one of the most adequate and sensitive bioindicators, relating to soil quality, fertility (Wolińska and Stępniewska, 2012) and overall microbial activity (Salazar *et al*., 2011), because dehydrogenases occur intracellular in all living microbial cells (Wolińska and Stępniewska, 2012). DHA reflects metabolic ability of the soil and its activity is considered to be proportional to the biomass of the microorganisms in soil (Wolińska and Stępniewska, 2012).

The objective of this experiment was to study the effect of fertilization on dehydrogenase activity in grassland soil. We hypothesized that the surface application of fertilizers on the grassland does not have strong and rapid impact on soil enzymatic activity.

Materials and methods

The experiment was conducted at the Estonian University of Life Sciences (58° 23' 32" N 26° 41' 31" E; elevation 60 m) in the year 2013 on a *Stagnic Luvisol* (WRB). The sward consisted of bluegrass (*Poa pratensis*) and red fescue (*Festuca rubra* L.). Treatments were: (i) control (no fertilizer was applied), (ii) mineral N-fertilizer (NH_4NO_3), (iii) cattle slurry, and (iv) cattle slurry digestate, in three replicates. Fertilizers were applied to the soil in quantities according to the nitrogen rate of 180 kg ha^{-1} in three equal splits on 3 May, 11 June and 30 July. The organic fertilizers application rate was calculated based on NH_4-N content. Fertilizers were surface-applied. The soil was sampled throughout the growing period: one day after fertilizer application and one and two months after the third fertilizer application. Soil samples (50 g)

were taken with a soil auger at depth 10 cm and stored in a refrigerator at 4ºC. Dehydrogenase activity was determined according to Von Mersi and Schinner (1991) in triplicate per sample and expressed by the dry weight of soil. Samples were incubated with iodonitrotetrazolium chloride (INT) and the formation of iodonitrotetrazolium formazan (INTF) was measured colorimetrically.

All calculations were performed using the statistical package Statistica 9.0 (StatSoft.Inc). The probability level was set at 0.05.

Results and discussion

Our results showed that the fertilization effect on DHA in grassland soil was not statistically significant ($P = 0.19$). The effect of organic fertilizers on DHA did not differ significantly from the effect of mineral fertilizer, although in the soil fertilized with organic fertilizers, DHA after the treatment was slightly higher than in the control and with the use of mineral – N, and 30 days after fertilization DHA in treatments with organic fertilizer was significantly ($P < 0.05$) higher when compared to the control (Table 1). In treatments with mineral-N, DHA was similar to the control or slightly lower. Earlier studies have shown highest DHA in soils treated with animal manure and the lowest in the unfertilized soil or in soil treated with mineral fertilizer (Parham *et al*., 2002) because addition of organic matter to the soil with organic fertilizers increases its microbial growth (Mijangos *et al*., 2006).

Our study showed that organic fertilizer effect on DHA in grassland soil (averaged for $0 - 10$ cm layer) was not significantly higher when compared to control and mineral fertilizer application. We speculate that this may be caused by the fertilizer application being on the surface of the grassland; therefore its influence did not reach deeper soil layers or the one day that remained between fertilization and sampling time was too short a period for the effect of fertilization to occur.

We found that DHA depended more on air temperature than on fertilization and fertilizer type (r = 0.48, $P < 0.05$), and it increased in all treatments with increase in the temperature. Dehydrogenase enzyme exists only inside the viable microbial cells; therefore, its activity should be the highest at a temperature close to the optimum temperature (30 °C) for microorganism growth (Wolińska and Stępniewska, 2011). As a result, DHA was the highest at the time of third fertilization ($P < 0.01$), when air temperature was the closest (21 °C) to the optimum temperature for growth of microorganisms. There was no significant difference in DHA between the first two fertilization times; then the mean air temperatures were respectively 11.9 °C and 13.8 °C.

Table 1. The effect of fertilization on DHA (µg INTF g^{-1}DM h^{-1}±se)

Treatment	Time of fertilization			Fertilizer aftereffect since the third application	
	3 May	11 June	30 July	30 days later	60 days later
Control	54.3 ± 10.7	61.4 ± 6.2	117.6 ± 21,6	61.1 ± 14.4	100.5 ± 25.2
Mineral N	67.1 ± 7.4	51.0 ± 5,8	102.0 ± 12.8	87.2 ± 14.3	83.9 ± 0.3
Digestate	68.9 ± 3.8	71.1 ± 8,1	145.3 ± 5.3	122.4 ± 8.6	103.9 ± 2.8
Cattle slurry	77.5 ± 19.2	76.0 ± 10.2	138.5 ± 10.8	118.3 ±19.6	97.1 ± 9.0

Conclusion

From our results it can be concluded that fertilization does not significantly affect microbial activity in grassland. More than fertilization and fertilizer type, the dehydrogenase activity depended on air temperature and it increased with the increase in temperature.

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Reduction of soft rush (*Juncus effusus L.*) by a combination of trimming and grazing

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Abstract

In wet grassland, soft rush (*Juncus effusus* L.) may be very dominant. This can make it difficult to obtain botanical diversity and a suitable habitat for meadow birds. When the swards are managed by grazing alone the soft rush is difficult to control because the animals have a low preference for this species. In this study different trimming strategies were compared in wet grassland grazed by young heifers and steers from dairy cattle. The four strategies were: 1) trimming in late April and in mid-October (early and late); 2) trimming late in April (early); 3) trimming in mid-October (late); 4) no trimming (none). No trimming and grazing resulted in an increase of soft rush measured on a DM-basis. The best effect was obtained with two trimmings a year, late April and mid-October. Management should be adjusted when the soft rush is reduced and the sward has become attractive to meadow birds.

Keywords: wet grassland, cutting, management, peat soil, biodiversity

Introduction

On grasslands, ecosystem services include delivery of botanical diversity and habitats for meadow birds. On moist soil it may be a problem to maintain botanical diversity and short vegetation for meadow birds in swards dominated by soft rush. Many of these grasslands are managed by cattle grazing, but when soft rush is dominating, the forage quality can be very low. Among the common species on moist grassland in Denmark the lowest digestibility was found for soft rush with an IVOMD value of 384 in July. At the same time, IVOMD of many of the other herbs varied between 600-800 (Nielsen and Søgaard, 2000). Comparing the effect of two cuts a year (middle of July and beginning of September) with the effect of continuous summer grazing with young dairy cattle, through a five-year period and leaving the vegetation to a cut in the middle of July in the sixth year, showed three times as much soft rush in the grazed sward than in the cut sward (i.e. 62% vs. 20% on DM basis) (Buttenschøn and Nielsen, 2004). Therefore, it seems possible to control the soft rush dominance by cutting, and farmers may obtain a more balanced diet for cattle if they combine grazing with trimming. To comply with Danish rules for farmers funded to manage grass- and nature areas, there is a requirement for no mowing between 1 May and 20 June; thus, early trimming for rush can be chosen in late April. Trimming in October can be chosen to prevent growth of the evergreen soft rush and make it vulnerable to the influence of water through winter. This paper presents results from a three-year study on the effect on soft rush of combining different strategies of trimming in paddocks grazed with young cattle from a dairy farm.

Materials and methods

The paddocks on permanent grassland used for the experiment were located at Fussingø Manor in Denmark. Four paddocks, 4 ha each, were grazed by heifers or steers of Holstein Friesian, or similar dairy cattle. Grazing started in the middle of May with a stocking density of approximately 750 kg per ha. From August to October cattle were withdrawn successively according to the decrease in grass production. Each paddock was located at a riverside area with most of the sward on moist peat soil, but about 20% of each paddock was on higher and dry mineral soil. Soft rush occurred only on the moist part of the paddock, and here four

different strategies of trimming were compared: 1) trimming in late April and in mid-October (early and late); 2) trimming late in April (early); 3) trimming in mid-October (late); 4) no trimming (none). Trimming was carried out with a rough cutter pulled by an all-terrain vehicle (ATV) with twin wheels. In each paddock, in the moist part, four plots of 10m × 10m were laid out at fixed points for botanical analysis. They were trimmed in the same way as the rest of the paddock. Before trimming in April samples were taken by cutting to the same height as trimming (7 cm) at 10×1 m^2. Representative subsample were sorted into living and dead biomass of soft rush, grass and other herbs. In May 2010 and 2012, botanical diversity was analysed using extended Raunkiær circles (Böcher and Bentzon, 1958) with three circles in each of the four plots per paddock. Compressed sward height was measured by a rising plate meter (30 × 30 cm; 3.8 kg m^{-2}) at specific routes in the paddocks, and the type of vegetation was recorded at each measurement. In addition to soft rush, the main other species in the sward were common species as *Festuca rubra*, *Poa trivialis*, *Holcus lanatus*, *Rumex acetosa*, *Ranunculus repens*, *Taraxacum sp.*, *Lotus pedunculatus var. pedunculatus*, *Trifolium repens*, *Juncus articulatus*, *Cirsium palustre*; as well as *Dactylorhiza majalis ssp. majalis*. During the years of the experiment the ground water level varied from 10 to 40 cm below surface in May-August, average around 20 cm. In autumn it varied from 5 to 20 cm below the surface, average around 10 cm.

Results and discussion

The results from compressed sward height showed that height in areas with pure grass was significantly lower than in similar areas of mixed grass and soft rush, and areas dominated by soft rush had the highest compressed sward height (Figure 1). The results confirm the need of the cattle to graze the more digestible part of the vegetation.
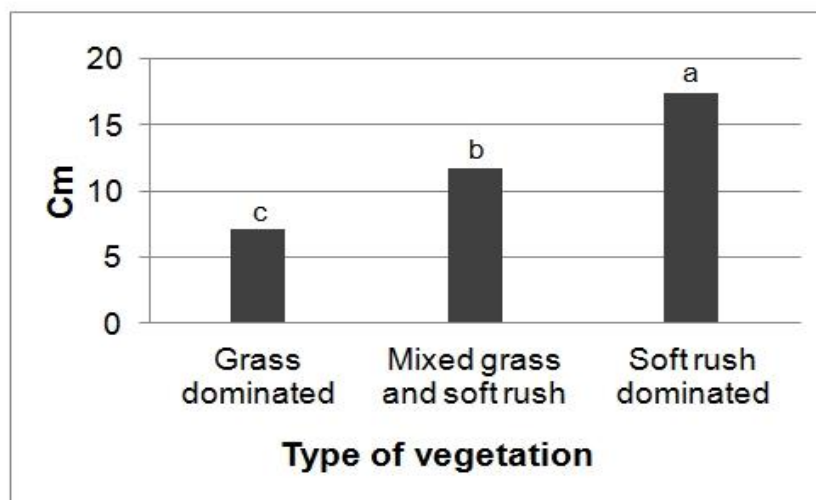
Figure 1. Sward height measured by rising plate meter in midsummer, average of measurements in the three years, 767 observations. Different letters indicate significant difference in height for type of vegetation ($P<0.001$).

Treatment effects on soft rush performance over years is shown in Figure 2. Statistical evaluation over years within the individual treatments showed that soft rush increased where no trimming was applied, and decreased by two yearly trimmings. The results from Raunkiær analyses showed that treatments did not change botanical diversity significantly over the relatively short period of the experiment (values not shown).
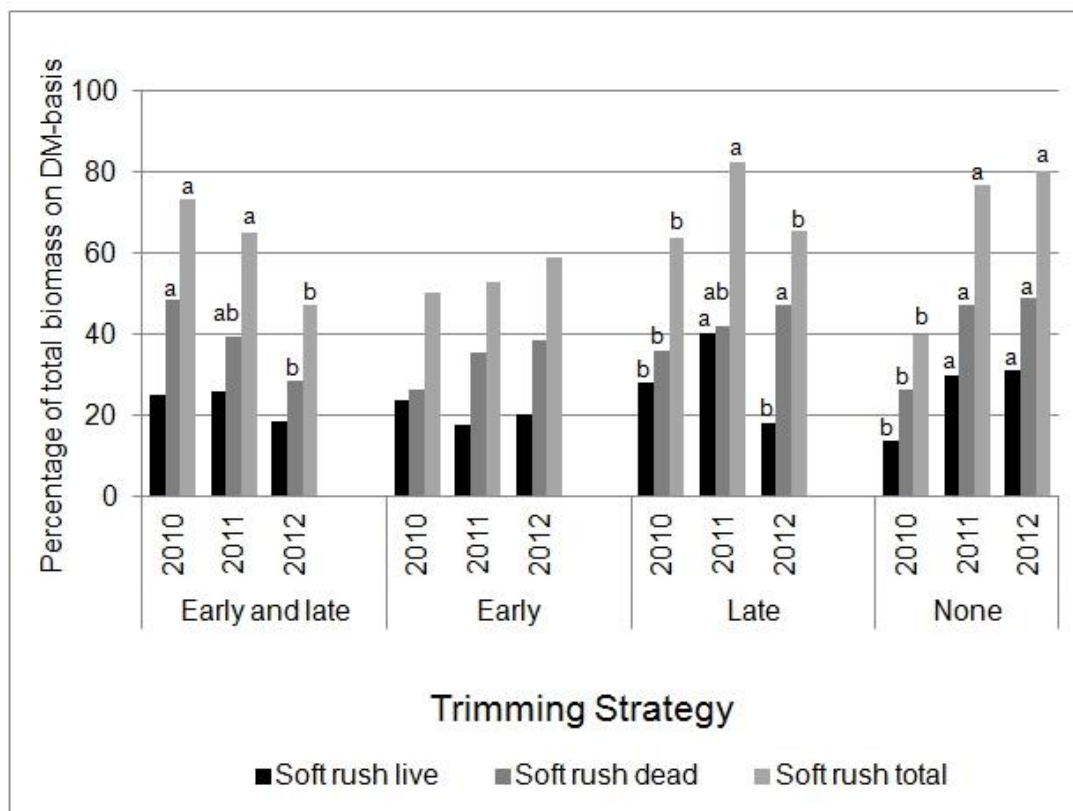
Figure 2. Percentage soft rush in swards managed with grazing and trimming, and the effect of trimming strategy on the soft rush content measured on a DM basis. Different letters indicate significant difference over year within strategy and type of biomass (*P*<0.05).

Conclusions

With no trimming, soft rush increased, as measured on a DM basis. The best effect of trimming was obtained with two trimmings a year: in late April and middle of October. When the soft rush is reduced the swards will become increasingly interesting for meadow birds, which should be considered in the future trimming strategy.

Acknowledgements

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Prospects for biological control of *Rumex obtusifolius* using a native clearwing moth

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Abstract

Broad-leaved dock (dock, *Rumex obtusifolius*) is among the most troublesome weeds in European grasslands and efficient non-chemical methods for its control are lacking. This study evaluated the ability of the native specialist insect *Pyropteron chrysidiforme* to attack dock using a mass release approach. Larvae of this moth mine into the roots of dock, which can negatively affect plant performance and induce mortality. At four agricultural grassland sites across Switzerland we applied insects at various developmental stages and levels of protection (eggs, larvae unprotected, larvae encapsulated) onto marked plants and examined the resulting infestation. High infestation rates were attained with the application of eggs (71% of plants infested), while larval applications were less successful. Moreover, differences among sites suggest that weather conditions and biotic interactions may influence the infestation success. These results show that successful infestation of dock by *P. chrysidiforme* can be attained under various conditions in agricultural grasslands, which is promising for biological control. However, higher infestation rates may be necessary for practical application and could be achieved by specific application techniques that reduce adverse environmental effects. Moreover, ongoing long-term field experiments are needed to evaluate the impacts of this method and its efficiency in controlling dock.

Keywords: biological control, *Pyropteron chrysidiforme*, root herbivory, *Rumex obtusifolius*, specialist insects, weed

Introduction

Broad-leafed dock (dock, *Rumex obtusifolius*) is among the most problematic weeds in agricultural grasslands and, because it is avoided by cattle, may lower fodder quality and rapidly displace other plants (Cavers and Harper, 1964). In addition to specific grassland management practices to reduce the presence of dock (Hopkins and Johnson, 2002), direct control approaches are used, which often include the use of herbicides. In order to reduce concomitant adverse effects for the environment and health, non-chemical alternatives are needed (Zaller, 2004). However, several attempts of above-ground biological control of dock have been deemed ineffective, given the plant's highly regenerative capacity from the roots. Therefore, a more promising approach may adopt the use of specialized root-feeding insects. This strategy was successfully implemented in a biological control programme using a Moroccan clearwing moth (*Pyropteron doryliforme*) against invasive dock species in Australia (Faithful, 2000). Larvae of these moths mine into the roots of dock and thereby negatively affect plant performance. In the framework of the Australian programme, a high potential to control dock was also attributed to the native European specialist root-feeding clearwing moth *Pyropteron chrysidiforme* (Scott and Sagliocco, 1991). To assess whether *P. chrysidiforme* may be a potential candidate for biological control of dock in Europe, we evaluated its capacity to attack dock under natural conditions in agricultural fields using a mass release approach. Specifically, we investigated the variations in infestation of *P. chrysidiforme* on dock following application of insects at various developmental stages and levels of protection (eggs, larvae

unprotected, larvae encapsulated). Additionally, we examined the potential influence of root biomass, vegetation density, and other site conditions.

Materials and methods

We conducted a field experiment at four agricultural grassland sites across Switzerland. In spring 2012, late-instar larvae of *P. chrysidiforme* were collected in natural populations in western Switzerland and subsequently reared in the lab to produce eggs and larvae. In June 2012, thirty *R. obtusifolius* plants per site (120 plants total) were permanently marked, and 10 plants per site were subjected to either application of eggs glued onto toothpicks (30 eggs / toothpick), transfer of unprotected larvae using a paintbrush (6 larvae / plant), or transfer of encapsulated larvae using metal syringes inserted into the root crown (6 larvae / plant). The four sites were regularly managed (cutting and/or grazing), except for a period of three to four weeks after the insect application, when the sites were protected from disturbance. From September to October 2012, we sequentially excavated the plants, determined root biomass and dissected the roots to record the infestation by larvae of *P. chrysidiforme*, which was evaluated as the percentage of plants infested and the number of larvae per infested plant. Differences in infestation among application techniques and sites as well as potential effects of vegetation density, root biomass, and harvesting dates, were analysed by mixed effects models using logit and poisson link (for infestation rate and number of larvae, respectively) with the function 'lmer' in the package 'lme4' (Bates, Maechler and Bolker, 2012) in R version 2.14.1 (R Development Core Team, 2013).

Results and discussion

On average, 54% of the treated plants were infested with at least one larva per root, which indicates that successful establishment of *P. chrysidiforme* on dock in agricultural grasslands can be achieved. Furthermore, infestation rates differed among application techniques (χ^2=7.197, df=2, $P<0.027$). Application of eggs resulted in higher infestation rates (71% of plants infested) compared to unprotected larvae (47%; $\chi^2=4.508$, df=1, $P_{(eggs\ vs.\ unprotected\ larvae)} < 0.034$) and encapsulated larvae (44%; $\chi^2=5.710$, df=1, $P_{(eggs\ vs.\ encapsulated\ larvae)} < 0.017$). One factor that may at least partly explain the difference between the egg and unprotected larval applications is predation of larvae by ants, which is likely to have less influence on smaller, newly hatched larvae from eggs (Pedrotta *et al*., unpublished results).

Furthermore, we found significant differences in infestation rates among the four experimental sites ($\chi^2=13.741$, df=3, $P<0.003$; Figure 1). Aside from different biotic interactions (i.e. predation by ants), other site conditions may also affect infestation success. In particular, the weather conditions at the time of insect applications at each site may be important to assure benign conditions for the larvae, as is shown in a complementary common garden experiment (Hahn *et al*., unpublished results). Compared to the transfer of unprotected larvae, application of eggs maybe generally be less prone to adverse environmental factors, because the higher number of larvae hatching from eggs and the longer time period in which hatching occurs may increase the chance of successful establishment of the larvae in the roots. The reasons for the low infestation rates associated with the encapsulated larval transfers are less clear; while encapsulation is expected to reduce adverse environmental effects, larvae may possibly suffer from the handling procedure. Other experimental factors such as root biomass and vegetation density did not affect infestation rates.
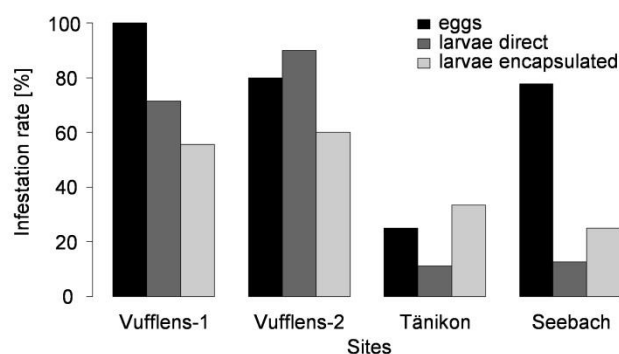
Figure 1. Proportion of *R. obtusifolius* plants infested with *P. chrysidiforme* at the four experimental sites, as a result of application of eggs or unprotected or encapsulated larvae.

In contrast to the number of infested plants, the number of larvae per root did not significantly differ among treatments and sites, and was neither significantly affected by root biomass, nor by vegetation density. On average, an infested root hosted 1.89 larvae of *P. chrysidiforme*, with an observed maximum of 5 larvae per root. This relatively constant number of larvae per root is consistent with previous studies, which reported about one larva per root (Scott and Sagliocco, 1991) and may be due to intra-specific competition. Nevertheless, observations have been made of more than 10 *P. chrysidiforme* larvae per root in natural infestations (Hahn, personal observation) and, therefore, other adverse environmental factors may also limit larval establishment in the roots.

Conclusions

Our study revealed that successful infestation of dock by *P. chrysidiforme* can be attained under various conditions in agricultural grasslands. This result is promising for the use of mass release of *P. chrysidiforme* for the control of dock. However, greater and more persistent infestation may be necessary for practical application. This could be achieved by specific application techniques that minimize adverse environmental effects. Moreover, ongoing long-term field experiments are needed to evaluate the impact of this method on plant and population performance over multiple years and thus, its efficiency in controlling dock.

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Effect of different grazing regimes on the coverage of *Taraxacum* spp. under a long-term grazing experiment

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Abstract

The effect of grazing by heifers under different grazing managements on the cover of *Taraxacum* spp. was studied on an upland long-term grazing experiment in the Jizerské hory Mts. from 1998 to 2013 The following treatments were applied: (i) intensive grazing (IG); (ii) extensive grazing (EG); (iii) first cut in the late spring and intensive grazing aftermath (ICG); (iv) first cut in the late spring and extensive grazing aftermath (ECG) and (v) unmanaged grassland (U) as the reference area. A significant effect of treatment, year and interaction of treatment and year on the % cover of *Taraxacum* spp. was revealed. The highest % cover was recorded in the ICG treatment in 2013. The combination of first cut and grazing the aftermath (ICG, ECG) strongly promoted the % cover of *Taraxacum* spp. up to 2004. After that there was a slight increase of *Taraxacum* spp. cover under the ICG and IG treatments. The highest % cover of *Taraxacum* spp. during the whole experiment was in the ICG treatment in 2013. Extensification of grassland management could be used as a simple tool for reduction of *Taraxacum* spp.

Keywords: heifer grazing, dandelion, cutting, management control

Introduction

Taraxacum spp. (Asteraceae) is a stemless perennial herb, native in Europe, and occupying a wide range of habitats especially pastures, lawns and meadows (Stewart-Wade *et al*., 2002). High plasticity and ecotype differentiation in ecophysiological traits allow this species to spread along wide environmental gradients for what it is considered to be one of the most aggressive invasive plants around the world (Molina-Montenegro *et al*., 2013). Although dandelion is classified as a serious agricultural weed in arable fields, its weediness in grassland is not so straightforward, because its high nutritive value and palatability (Marten *et al*., 1987) can increase the quality of pasture forage (Pavlů *et al*., 2006). The objective of the study reported in this paper is to evaluate the effect of different grazing intensities on the cover of the dandelion and to recommend which management can be used for dandelion control in grasslands.

Materials and methods

The study site was performed on experimental grassland in the Jizerské hory Mountains, 10 km north of the city of Liberec, Czech Republic. The long-term grazing experiment called 'Oldřichov Grazing Experimen' (OGE) was established in the spring of 1998 and was arranged in two randomized blocks (Pavlů *et al*., 2007). The following treatments were studied: intensive grazing (IG), first cut in the late spring and intensive grazing aftermath (ICG), extensive grazing (EG), first cut in the late spring and extensive grazing aftermath (ECG), and unmanaged grassland (U) as the reference area.

Development of dandelion % cover was recorded in permanent 1 m × 1 m plots using a continuous grid of nine 0.33 m × 0.33 m subplots in four replications in each paddock. Dandelion covers in each subplots were visually estimated in early May each year from 1998 to 2013. The mean of nine subplots was used for statistical evaluation. Repeated measures

ANOVA was used to evaluate seasonal development of the cover of *Taraxacum* spp. One-way ANOVA was used to test the cover of *Taraxacum* spp. in the particular year.

Results

A significant effect of treatment, year, and interaction of treatment and year, on the cover of *Taraxacum* spp. was revealed (Table 1).

Table 1 Results of repeated measurements ANOVA analyses for seasonal development of % cover of *Taraxacum* spp. for years 1998 – 2013.

Taraxacum spp.	Effect	Degree of freedom	*F*-ratio	*P*-value
% cover	Treatment	4	126.5	<0.001
	Year	15	15.8	<0.001
	Treatment × Year	60	3.6	<0.001

Immediately after management was imposed in 1998 all types defoliation treatments supported an increase of *Taraxacum* spp. cover (Figure 1).
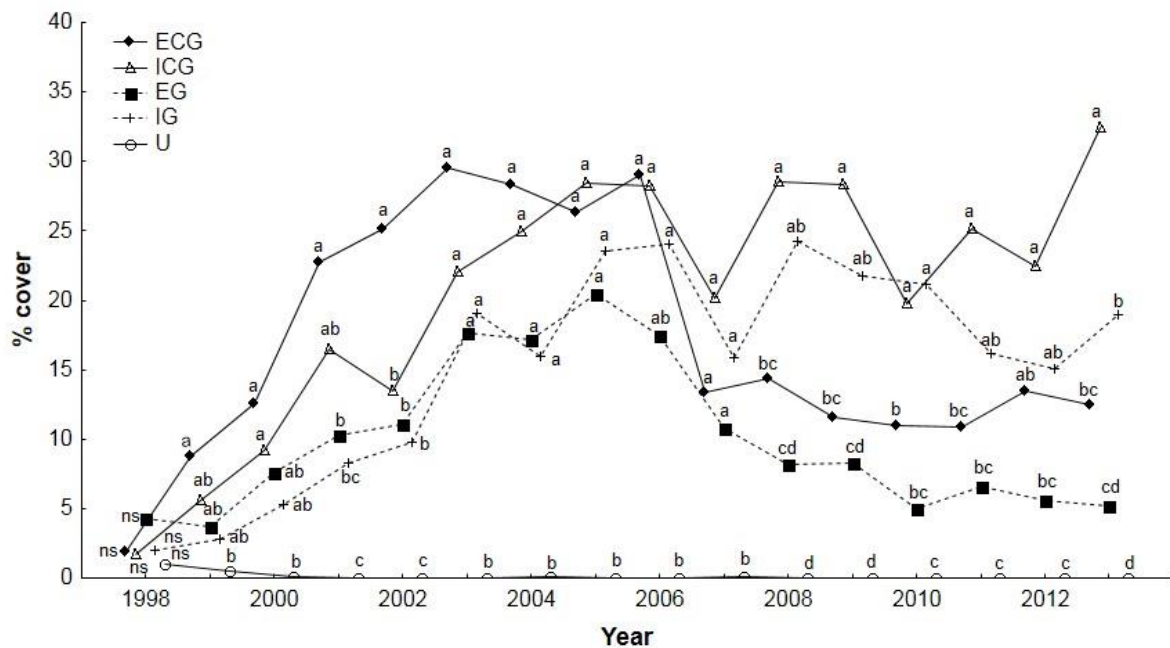
Figure 1. Changes in % cover of *Taraxacum* spp. under different treatments for the years 1998-2013. *P* represents probability value obtained by one-way ANOVA for each year and $P < 0.01$ was for all analyses, n.s. – non-significant result. Significant differences ($P < 0.05$) according to the Tukey post hoc test are indicated by different letters

The lowest cover was recorded in the unmanaged treatment (U) during the whole experiment. Up to 2004, *Taraxacum* spp. was promoted especially by the combination of cut and grazing aftermath (ICG, ECG); however, the differences among all managed treatments were small. In 2007 there was revealed a striking decrease of *Taraxacum* spp. in all defoliation plots. The highest decrease was recorded in the ECG treatment (from 29% to 13%), followed by IG and ICG (a decrease about 8%) and EG (7%). After that a slight increase of *Taraxacum* spp. cover was revealed under the ICG and IG treatments. The highest cover of *Taraxacum* spp. during the whole experiment was 32%, in the ICG treatment in 2013.

Discussion

The higher defoliation intensity under intensive grazing is more favourable for dandelion presence. Sparse canopy and more light at the soil surface under intensive management increase the possibility for germination of dispersed diaspores and of plants succeeding into the generative phase (Pykälä, 2005). Similarly, the most favourable treatment for *Taraxacum* spp. presence in the course of our experiment was that of cutting in late spring and intensive grazing aftermath. It shows that, in Central European conditions, the applied cutting management in late May or early June can allow dandelion to reproduce and consequently spread its seeds. For that reason early cutting seems to be an appropriate method for range management to control its dispersal and germination of seedlings (Martinkova *et al.*, 2009).

Conclusion

Intensive grazing was the treatment that most promoted the abundance of *Taraxacum* spp. and this effect was strengthened by cutting in the late-flowering period. Therefore, an early cut at the beginning of the *Taraxacum* flowering period could lead to elimination of flowers and thus to reduced seed production. Extensification of grassland management could be used as a simple tool for decreasing the presence of *Taraxacum* spp.

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Emergence and survival of *Rumex* OK-2 (*Rumex patientia* x *Rumex tianschanicus*) in grasslands under different management conditions

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Abstract

Emergence and survival of *Rumex* OK-2 was studied in the north of the Czech Republic (in an experimental garden in Liberec town) in 2013. Three frequencies of cutting were applied: no (0C), one (1C) and three (3C) cuts per year. Seeds of *Rumex* OK-2 were sown into the sward with different microsite conditions (no gap − gap; fertilizer application and no fertilizer application) in each treatment. The following plant characteristics were measured: number of emerged plants, number of surviving plants, plant height and numbers of leaves. Measurements were made three times per vegetation season (middle of June, end of July and end of September) before cutting. Plants of *Rumex* OK-2 emerged more in the treatments with gaps. Survival of *Rumex* OK-2 plants was connected with treatments with gap, especially in the second and the third cutting date.

Keywords: weeds, cutting frequency, competition, fertilizers application, gap

Introduction

Many broad-leaved *Rumex* species are considered to be the most troublesome weeds in grasslands and arable land worldwide (Zaller, 2004). These plants often colonize grasslands as well as permanent agricultural crops (Novák, 1994; Brant *et al.*, 2006), where they can survive for a long time (Martinková *et al.*, 2009). A new forage and energy-crop hybrid, *R. patienta* × *R. tianschanicus*, registered as cv. *Rumex* OK-2 (hereafter referred as *Rumex* OK-2) was introduced into the Czech Republic about ten years ago (Usťak, 2007). *Rumex* OK-2 is described as a perennial (up to 10 years) stress-tolerant plant, characterized by high ecological plasticity, with cold and winter hardiness, tolerance to salt stress and increased humidity (Kosakivska *et al.*, 2008). Before the introduction to the culture it was presented as a competitive species with a low possibility of invasibility (Usťak, 2007). However, it behaves as an invasive weed species, especially in road ditches covered by grasslands in the vicinity of the field where it was previously grown (Hujerová, 2013a). The response of mature plants to different cutting frequencies of *Rumex* OK-2 is very similar to that of *Rumex crispus* (Hujerová 2013b). In view of the above-mentioned knowledge we established a manipulative experiment where emergence and survival of *Rumex* OK-2 in grasslands under different management conditions were studied.

Methods and materials

A plot experiment was conducted in 2013 at the experimental garden of the Crop Research Institute, Grassland Research Station Liberec, in the northern part of the Czech Republic, under conditions of natural rainfall, temperature and daylight. Twenty seeds of *Rumex* OK-2 were sown into the sward in May 2013. Twelve factorial treatments were applied: i) three frequencies of cutting- no (0C), one (1C) and three (3C) cuts per year; ii) two levels of disturbance - gap and no gap; iii) two levels of nutrients - fertilizers application and no fertilizer application. The experiment was arranged in four complete randomized blocks with individual plot sizes of 0.5 m × 0.5 m. NPK fertilizer was applied in amounts of 100 kg N ha⁻¹ 52 kg K

ha^{-1} and 27 kg P ha^{-1} in 0 15 m × 0.15 m areas allocated in the middle of each plot. Seeds were sown in the same area. We recorded number of emerged plants, number of surviving plants, plant height and numbers of leaves. Measurements were made three times per season (middle of June, end of July and September) before cutting. In the first cutting term the *Rumex* plants were not defoliated, because they were smaller than cutting height. One-way ANOVA and repeated measures ANOVA were used to evaluate number of emerged plants, number of surviving plants, plant height and numbers of leaves.

Results and discussion

The number of emerged *Rumex* OK-2 plants was significantly divided into two groups according to disturbance. In the treatments without gap, up to one plant per plot was found, whereas in plots with gap there were from seven to nine plants after one-and-a-half months after sowing date (Figure1).
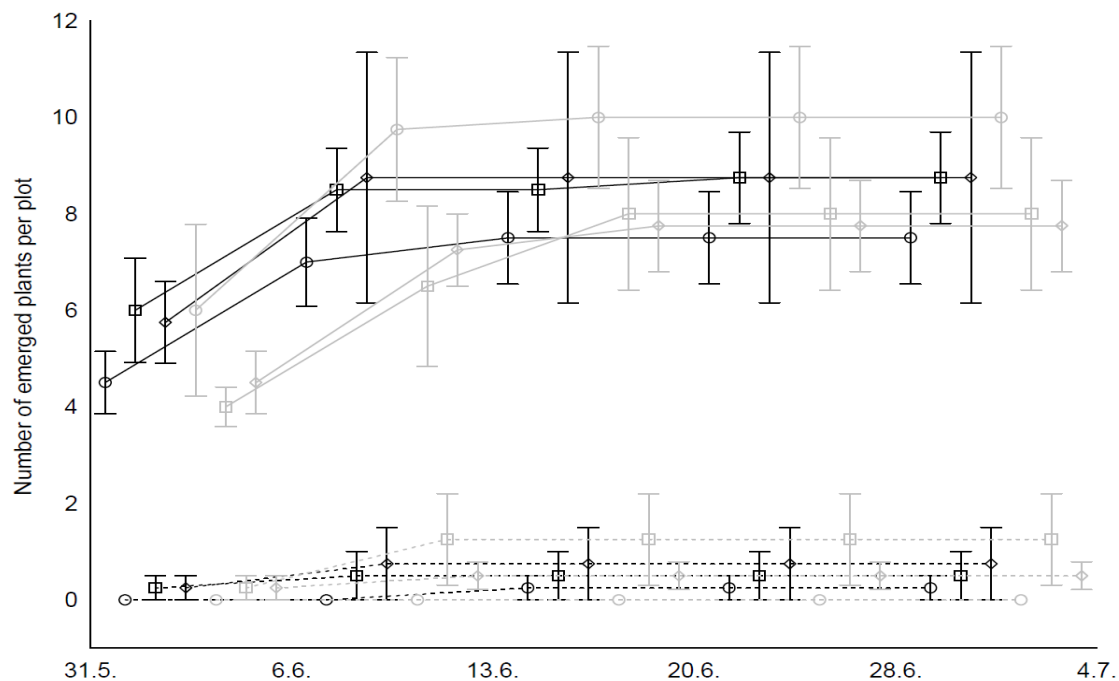
Figure 1. Development of emerged *Rumex* OK-2 plants during one-and-a-half months after sowing.
Legend: ■■■ fertilized, ▬▬▬ non-fertilized, ○ 0C, □ 1C, ◇ 3C, ── gap, --- no gap.

There were no emerged plants found in the no-gap, non-fertilized, no-cutting treatment (NGaNFC0). On the other hand the highest number of emerged plants was in the gap, non-fertilized, no cutting treatment (GaNFC0). It confirmed the results of Carvers and Harper (1964) for *Rumex crispus* and *R. obtusifolius* that seed germination is possible when a gap occurs in the established sward. In the first cutting term there were only a few surviving plants in treatments without gaps but several times more of them in treatments with gaps (Figure 2). However, the number of surviving *Rumex* OK-2 plants significantly decreased in the second cutting term because of high competitive ability of the existing sward. After the third cut only a few of the *Rumex* OK-2 plants survived in gap treatments. However, due to its fast spring growth and similar tolerance to cutting as *R. crispus* has (Hujerová, 2013b) we can expect it surviving, with possible flowering and consequent seed production in the next vegetation.
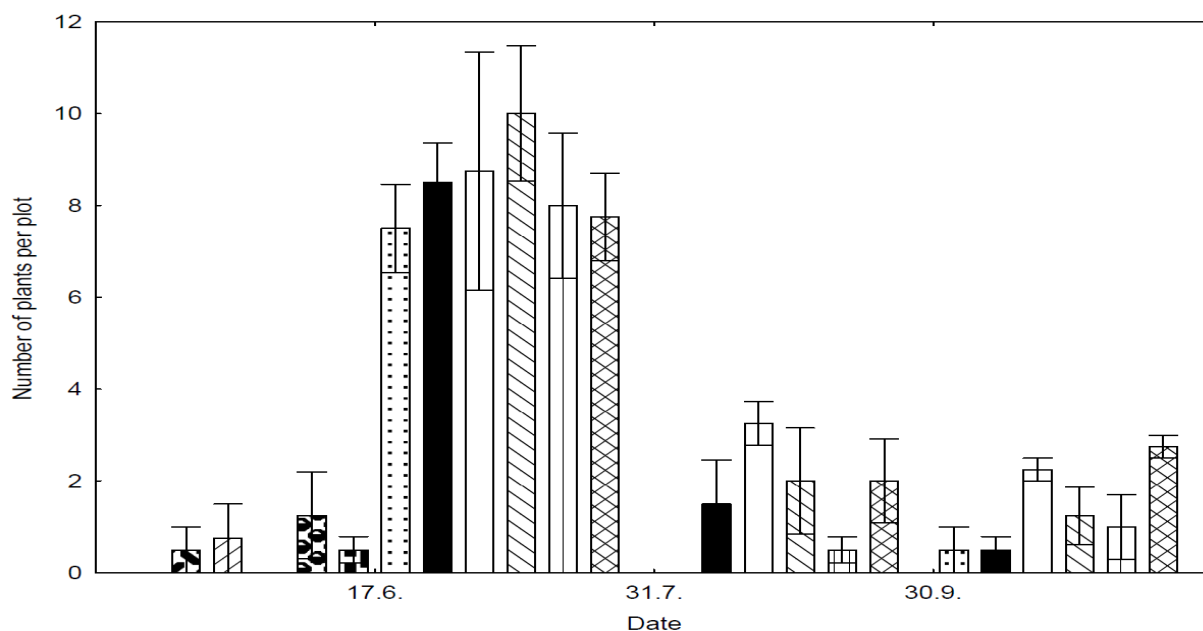
Figure 2. Number of survived plants *R*. OK-2 in three cutting terms. ▨NGaFC1, ▱NGaFC3, ◩NGaNFC1, ▦NGaNFC3, ⬚GaFC0, ■GaFC1, ☐ GaFC3, ⬔GaNFC0, ▥GaNFC1, ⊠GaNFC3.

Conclusion

Sward disturbance is the main factor for *Rumex* OK-2 infestation into existing grasslands. Although in the course of the vegetation season plants of *Rumex* OK-2 are exposed to high competitive pressure of existing sward, some were still revealed at the end of vegetation season. These plants in the next vegetation seasons can become an important source of seeds and support its expansion into the surroundings. *Rumex* OK-2 has similar behaviour as other broad-leaved docks in Central Europe, so we can expect its further spreading.

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Mixed cropping of grass and alfalfa to reduce weed growth

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Abstract

Alfalfa crops, as most pure-stand crops, often require herbicide treatments. The introduction of grass species grown in mixture with alfalfa has been studied in a micro-plot design located at two sites and harvested with an infrequent cutting schedule. Eight meadows were studied: seven alfalfa-grass mixtures (seven grass species tested) and one pure alfalfa. At each cut during three years, the weed proportion in harvested biomass was measured by manual sorting of species. The weed proportion reached up to 60% of the harvested biomass in pure alfalfa, but on the alfalfa-grass mixtures, it was reduced by 75% at one site and 90% at the other site on average in the first six cuts. There was little difference in the proportion of weeds between the different grass species. However, weed proportion tended to be lower with the most aggressive grass species (perennial ryegrass and festulolium) than with the other species. These results indicate that herbicide spraying could be greatly reduced if alfalfa-grass mixtures were cultivated instead of pure alfalfa. In addition, alfalfa-grass mixtures could have even greater advantage than pure alfalfa in cereal rotations to decrease weed pressure.

Keywords: alfalfa, grass, legume, mixture, weed

Introduction

Today, one of the challenges of agriculture is to reduce significantly the use of pesticides, particularly herbicides that contaminate rivers and aquifers. In addition, for some crops such as alfalfa (*Medicago sativa*), the successive withdrawals of approvals of active substances limit the chemical solutions for weed control. In a pure alfalfa stand grown without herbicide treatment at sowing, the proportion of weeds can represent a significant part of biomass during the first cuts (Spandl *et al*., 1999) and severely compete with the establishing alfalfa plants. With its erect growth habit and slow aerial growth after sowing or cutting, alfalfa does not cover between-row space during the establishment phase, in early spring or after a cut. These are key periods for the development of weeds in the canopy and their presence leads to losses in forage production and quality and may reduce crop persistency. The emergence of weed seedlings is related to the composition of the active seed bank available in the topsoil. Kruidhof *et al*. (2008) showed that, because of its limited ability to intercept the light radiation in the establishment phase, alfalfa was much less competitive with weeds than most grasses. The objective of this study was to determine whether the combination of grass species with alfalfa could significantly reduce the proportion of weeds in harvested forage. Several forage grass species with varying characteristics were tested.

Materials and methods

Two micro-plots (9 m²) trials consisting of binary alfalfa-grass mixtures were sown on two contrasting sites: Somme-Vesle (in summer 2006) in the North-East of France and Lusignan (in summer 2007) in Centre-West of France. The soil in Somme-Vesle is a shallow sandy loam with a pH of 8.3, while in Lusignan it is a deep sandy clay loam with a pH of 6.5. The seven grass-alfalfa mixtures are shown in Table 1. A pure alfalfa (ALF) was included in the

Table 1. List of binary alfalfa-grass mixtures and weight of seeds sown for each species (kg/ha).

Species	Grass species associated to alfalfa	Variety	Weight of seeds	
			Grass	Alfalfa
ALF-TF	Tall fescue (*Festuca arundinacea*)	Flexy	8.9	14.5
ALF-OG	Orchardgrass (*Dactylis glomerata*)	Lupre	7.3	14.5
ALF-MF	Meadow fescue (*Festuca pratensis*)	Préval	7.3	14.5
ALF-AB	Alaska brome (*Bromus sitchensis*)	Hakari	13.2	14.5
ALF-TI	Timothy (*Phleum pratense*)	Barfleo	3.0	14.5
ALF-FL	Festulolium (*Festuca glaucescens x Lolium multiflorum*)	Lueur	11.0	14.5
ALF-RG	Ryegrass (*Lolium perenne*)	Brest	11.0	14.5
Species in pure stand				
ALF	Alfalfa (*Medicago sativa*)	Comete	-	22.0

trials as a control. Sowing densities were defined to achieve an equal number of seeds per unit area of both components of binary mixtures and among mixtures. Sowing density of controls are the common practices. A phospho-potassium fertilization was applied during the winter that followed the sowing, with 600 kg/ha of K_2O and 180 kg/ha P_2O_5. No nitrogen fertilization was applied and no weed control was done. Plots were harvested with a forage harvester (Haldrup) and cutting occurred approximately after 42 days of regrowth. The proportion of weeds in the plots was determined from a sample of about 500 g of fresh biomass at harvest time. All species were manually separated (alfalfa, grass, weeds), dried at 60 °C for 72 hours and weighed to determine the proportion of each species as a percentage of total dry matter. The data were subjected to analysis of variance with Statistica software and the results were compared by a Student-Newman-Keuls test.

Results and discussion

In Somme-Vesle, the pressure of the weed flora was high. The proportion of weeds in the harvested biomass averaged 5.8% but reached 61% (Table 2). In each year, the weed proportions were higher in the first spring cut than in the next cuts, with 24.6, 13.1 and 9.1% for the first, second and third year, respectively. In Lusignan, weed pressure was low. The weed proportion in the harvested biomass averaged 1.5% and reached a maximum of 18%. There was little difference between the first spring cuts and the other cuts. At both sites, the weed proportion was significantly higher in the pure alfalfa stand than in the alfalfa-grass mixtures in the four cuts of the first year and the first two cuts of the second year, but not in the next cuts of the second and third years. In these six first cuts, the weed proportion in the mixtures, compared to pure alfalfa, was reduced by 44 to 90% in Somme-Vesle and by 79 to 98% in Lusignan, depending on the grass species. Even if the weed proportion was low in the mixtures, there was difference between them. Perennial ryegrass and festulolium were the two grass species that most limited the growth of weeds in the first six cuts at both sites, where the frequencies of weeds were the highest. Both grass species are known to establish quickly and are considered as aggressive. In contrast, the highest weed proportion in mixture was observed with brome in Somme-Vesle and timothy in Lusignan.

Table 2. Biomass yield (t dry matter/ha), weed proportion in the harvested biomass (%) in both sites and each cut (C) during the 3 years and significance of effects of grass species and block for each cut in analysis of variance.

Species	Year 1				Year 2				Year 3			
	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
Somme-Vesle												
Yield	*4.7*	*3.2*	*2.9*	*2.4*	*4.8*	*2.4*	*2.8*	*-*	*5.5*	*3.9*	*3.7*	*2.3*
ALF-TF	29.2ab	0.9a	-	0.0a	11.0a	0.2a	0	-	0.7a	0.0a	0.0a	0.0
ALF-OG	18.6ab	3.6a	-	0.0a	11.3a	0.0a	0	-	6.5a	0.0a	0.0a	0.0
ALF-MF	20.7ab	1.9a	-	0.0a	0.7a	0.0a	0	-	4.5a	0.0a	0.0a	0.0
ALF-AB	34.7ab	6.5a	-	2.1a	34.3b	2.4b	0	-	27.6a	1.1a	0.0a	0.0
ALF-TI	26.1ab	3.8a	-	0.0a	8.5a	0.0a	0	-	8.9a	0.0a	1.1a	0.0
ALF-FL	10.1a	1.4a	-	0.0a	4.3a	0.0a	0	-	6.4a	4.2a	0.0a	0.0
ALF-RG	9.6a	0.2a	-	1.4a	3.5a	0.0a	0	-	8.4a	4.9a	0.3a	0.0
ALF	**61.3c**	**13.7b**	**-**	**15.0b**	**44.5b**	**7.6c**	**0**	**-**	**18.8a**	**0.0a**	**0.0a**	**0.0**
Anova												
Species	***	***	-	***	***	***	-	-	ns	ns	ns	-
Block	ns	ns	-	ns	ns	ns	-	-	ns	ns	ns	-

Species	Year 1				Year 2				Year 3			
	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
Lusignan												
Yield	*4.5*	*3.7*	*3.0*	*1.7*	*6.1*	*6.6*	*4.8*	*1.4*	*8.4*	*5.1*	*3.4*	*1.3*
ALF-TF	1.7a	0.3a	0.0a	0.0a	0.8a	0.3a	0.0a	0.0a	0.3a	0.0a	0.2a	0.0a
ALF-OG	0.9a	0.2a	0.3a	0.3a	0.7a	3.2a	0.0a	0.2a	0.9a	0.1a	0.2a	0.2a
ALF-MF	1.2a	0.3a	4.2a	0.2a	1.4a	1.5a	0.3a	0.1a	0.4a	3.3a	0.0a	0.0a
ALF-AB	0.7a	3.8a	1.3a	0.6a	6.4b	2.3a	0.0a	0.1a	0.9a	3.4a	0.1a	0.1a
ALF-TI	2.0a	2.0a	5.0a	2.0a	2.2a	0.7a	0.2a	0.0a	2.3a	0.8a	0.1a	0.1a
ALF-FL	0.0a	0.0a	0.0a	0.6a	1.2a	0.8a	0.5a	0.0a	1.6a	0.2a	0.2a	0.2a
ALF-RG	0.0a	0.2a	0.3a	0.3a	0.0a	0.5a	0.2a	0.3a	0.4a	0.0a	0.0a	0.6a
ALF	**10.3b**	**16.5b**	**17.6b**	**7.5b**	**8.7b**	**5.6a**	**0.1a**	**0.0a**	**0.2a**	**0.1a**	**0.0a**	**0.0a**
Anova												
Species	***	***	***	***	***	ns	ns	ns	ns	ns	ns	ns
Block	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Conclusion

In the context of limiting the use of pesticides, the combination of a grass with alfalfa can significantly reduce the weed proportion in harvested biomass. This type of mixture contributes to limit or even eliminate herbicide spraying during the establishment of alfalfa. The interest of alfalfa cropping in cereal rotations to limit the development of weeds in the following crops is mainly related to a change in weed flora (Meiss *et al*., 2010). With the strong reduction of weeds in the various years of the alfalfa-grass mixtures, our study shows that this type of grassland would further reduce weed pressure, in addition to a change in weed flora.

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Impact of site conditions on natural and fodder value of meadow-pasture communities with different contributions of *Urtica dioica* L.

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Abstract

The effect of habitat conditions on the occurrence of *Urtica dioica* and the resulting natural and fodder value of swards in meadow-pasture communities were assessed. Habitat conditions, i.e. water content, soil reaction, contents of available forms of phosphorus, potassium and magnesium as well as nitrate nitrogen were analysed using laboratory methods. Species richness, floristic diversity index and fodder-value score of the whole sward were determined in communities with different proportions of common nettle. The highest share of this species was found in well-watered habitats, on slightly acidic or acidic soils, abundant in nitrate nitrogen and low in magnesium and potassium. An increase in the contribution of *U. dioica* in the sward had a negative effect on its natural and fodder value.

Keywords: competitiveness, meadow communities, habitat, utilization

Introduction

The preservation of semi-natural grassland communities requires their regular and sustainable mowing or pasture use. Both intensive use with excessive fertilization, and also negligence of grassland use, contribute to transformations in their floristic composition, and thus to changes in their fodder value expressed in their palatability, digestibility and chemical composition (Trzaskoś, 1995; Kryszak *and* Kryszak, 2005). At present, the transformation of floristic composition mainly consists of changes in the proportions of individual species in the sward of meadows and pastures, connected, e.g., with encroachment of new species exhibiting high adaptability (Genovesi, 2004). An example may be provided here by common nettle (*Urtica dioica* L.), a species increasingly often found in high proportions and in large clusters in swards of permanent grassland. The aim of this study was to indicate habitat conditions promoting the occurrence of *Urtica dioica*. The effect of the contribution of this species on the natural and fodder value of swards in meadow-pasture communities was also determined.

Material and methods

Habitat conditions promoting the occurrence of *Urtica dioica* were determined using laboratory methods: soil moisture content by gravimetry (over-dry method), soil reaction by H_2O potentiometry, available potassium, phosphorus and magnesium forms were determined in 0.5 mol/dm^3 HCl extract, while the Kjeldahl method was used to determine contents of nitrate nitrogen (Boratyński *et al.*, 1988). Samples for chemical analyses were collected from a depth of 15-20 cm soil from canary grass, foxtail grass and soft grass meadows, ryegrass pastures and those with the dominance of Kentucky bluegrass and red fescue, differing in the share of common nettle: A − absent in the sward, B − < 1 % share in phytocenosis, C − > 1% share. Statistical analysis methods using Canoco for Windows 4.5 (ter Braak *and* Smilauer, 1997-2002) were applied in the analysis of results. In addition, based on the floristic composition described in 150 relevés of approximately 100 m^2 each, the mean number of species in the

phytocenoses was determined along with the floristic diversity index (Shannon-Wiener), and fodder value of the whole sward according to Filipek (1973).

Results and discussion

Results of the study indicate that the occurrence of *Urtica dioica* depends on soil reaction, and its proportion was found to increase with a decrease in soil pH. Moreover, $N-NO_3$ content influences the share of *Urtica dioica* in the sward of meadows and pastures. However, it is connected with habitat water contents. In habitats of well-watered canary grass meadows no marked dependence could be observed between the share of this species and $N-NO_3$ content in the soil, which is connected with a deficit of available nitrogen forms. In contrast, in less-moist or in dry habitats a marked dependence was found for an increase in the share of *Urtica dioica* with an increase in $N-NO_3$ content in the soil. These dependencies are confirmed by the analysis of distribution of variables presented in the diagram (Figure 1), indicating that the most important factors influencing the share of *Urtica dioica* include soil contents of nitrate nitrogen, water content and soil reaction. Similarly, in moderately moist habitats a trend was observed for an increase in the contribution of *Urtica dioica* with an increase in soil abundance of available phosphorus forms and a decrease in magnesium and potassium abundance.

Explanations:
A – without nettle
B – share of nettle < 1%
C – share of nettle > 1%
Ph – canary grass meadow
Al – foxtail meadows
Ho – *Holcus* meadows
Lo – ryegrass pasture sward
Pp-Fr – pasture sward with *Poa pratensis* and *Festuca rubra*
M – moisture content
pH – soil pH in H_2O
Mg – absorbable form of magnesium $(mg \cdot kg^{-1})$
K – absorbable form of potassium $(mg \cdot kg^{-1})$
P - absorbable form of phosphorus $(mg \cdot kg^{-1})$
N-NO3 – nitrate nitrogen $(mg \cdot dm^{-3})$

Figure 1. Habitat diversity of plant communities depends on the share of common nettle

Results of this study confirm the effect of the share of *Urtica dioica* on natural and fodder value of swards in the examined communities. In all the communities with a low proportion of *Urtica dioica* an increase was observed in the mean number of species, which was accompanied by an increase in floristic diversity manifested in the calculated values of the Shannon-Wiener index (Table 1). In contrast, an opposite relationship was observed at an increase in the share of this species (Table 1). Some authors explain this phenomenon by high competitiveness of *Urtica dioica* (Grime *et al.*, 2007).

Moreover, participation of *Urtica dioica* in the sward exceeding 1% results in a reduction of shares of grass species with high feeding value, such as *Lolium perenne*, *Poa pratensis*, *Phleum pratense*, *Alopecurus pratensis* and *Arrhenatherum elatius*, causing at the same time a deterioration of fodder value. A significant reduction of sward fodder value was observed in

foxtail grass meadows, where the fodder value index dropped from 7.51 (good sward) to 5.72 (poor sward).

Table 1. Natural and useful characteristics of studied plant community

Community and utilization		Number of plant species, average in releves	H'*	FVS **
Canary grass meadows 2-3 x cuting	A	21	1.7	5.75
	B	24	1.8	6.18
	C	15	1.6	4.57
Foxtail meadows 2-3 x cuting	A	17	1.4	7.51
	B	20	1.9	8.12
	C	16	1.4	5.72
Holcus meadows 1-2 x cuting	A	23	1.8	5.12
	B	25	1.9	5.31
	C	18	1.7	4.82
Ryegrass pasture sward pasturage/grazing: 2-3 LU ha^{-1}	A	19	1.8	8.09
	B	22	1.8	8.29
	C	23	2.1	7.25
Pasture sward with *Poa pratensis* and *Festuca rubra* pasturage/grazing: 1 LU ha^{-1}	A	18	2.0	6.31
	B	22	2.1	6.74
	C	20	2.0	6.16

* H' - floristic diversity index- according to Shannon-Wiener; ** FVS -fodder value score - (Filipek, 1973).

Conclusion

The occurrence of common nettle (*Urtica dioica*) is promoted by well-watered habitats, soils with slightly acidic or acid reaction, as well as high soil resources of nitrate nitrogen and low magnesium and potassium contents in soil. An increase in the share of common nettle in the sward has a negative effect on its natural and fodder value.

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Tree and pasture productivity in *Pseudotsuga menziesii* (Mirb.) Franco silvopastoral systems fertilized with sewage sludge

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Abstract

In Galician silvopastoral systems (northwest Spain) fertilization with sewage sludge could enhance tree and pasture productivity which is limited by soil acidity. The effect of sewage sludge on tree growth and the pasture production is different depending on the process used to stabilize the sewage sludge. The aim of the present study was to evaluate the effect of fertilization with municipal sewage sludge, which has been stabilized using anaerobic digestion, composting, and pelletization, on tree and pasture productivity compared to control treatments (mineral and no fertilization) in a silvopastoral system under *Pseudotsuga menziesii* (Mirb.) Franco. Mineral fertilization increased the annual pasture production and reduced the tree heights due to the competition by the nutrients generated between pasture and trees. However, tree height was increased by the application of pelletized sewage sludge applied in split doses.

Keywords: agroforestry, sowing, afforestation, anaerobic digestion, composting, pelletization

Introduction

In Spain, around 65% of sewage sludge is recycled through agricultural soils. This is due to its agronomic value as a source of plant nutrients and organic matter, and its soil-improving qualities (MMA, 2006). Sewage sludge could be used as fertilizer in Galician silvopastoral systems (northwest Spain) in which tree and pasture productivity is limited by low soil fertility as a result of increased acidity. In Europe, sewage sludge should be stabilized before being used as fertilizer. The stabilization process could cause differences in the mineralization rates (EPA, 1994) and, therefore, in tree growth and pasture production. Anaerobic digestion and composting are the most important types of sludge stabilization promoted by the EU (European Directive 86/278) (EU, 1986). However, both types of waste contain high proportions of water which could be reduced by 98% through pelletization of anaerobic sludge via thermic treatment; this reduction consequently reduces the storage, transport and spreading costs compared with anaerobic or composted sludge (Mosquera-Losada *et al*., 2010). The aim of the present study was to evaluate the effect of fertilization with municipal sewage sludge that has been stabilized using anaerobic digestion, composting, and pelletization, on tree and pasture productivity compared to control treatments (mineral and no fertilization) in a silvopastoral system under *Pseudotsuga menziesii* (Mirb.) Franco.

Materials and methods

The experiment was established in Baltar, A Pastoriza (Lugo, Galicia, northwest Spain) at an altitude of 475 m above sea level. Pasture was sown with a mixture of *Dactylis glomerata* L. var. Artabro (12.5 kg ha^{-1}), *Lolium perenne* L. var. Brigantia (12.5 kg ha^{-1}) and *Trifolium repens* L. var. Huia (4 kg ha^{-1}) in December 2004. Plants of *Pseudotsuga menziesii* (Mirb.) Franco were planted at a density of 952 trees ha^{-1} after pasture sowing in February 2005. The experimental design was a randomized complete block with three replicates and five treatments. Each experimental unit had an area of 168 m^2 and 25 trees planted with an arrangement of 5×5 stems, forming a perfect square. Treatments consisted of (a) no fertilization (NF); (b) mineral fertilization (MIN) with 500 kg ha^{-1} 8:24:16 compound fertilizer

(N:P_2O_5:K_2O) at the beginning of the growing season and 40 kg N ha^{-1} before first harvest; (c) fertilization with anaerobically digested sludge (ANA) with an input of 320 kg total N ha^{-1} before pasture sowing; (d) fertilization with composted sewage sludge (COM) with an input of 320 kg total N ha^{-1} before pasture sowing and (e) application of pelletized sewage sludge (PEL), which involves a contribution of 320 kg total N ha^{-1} split as 134 kg total N ha^{-1} just before pasture sowing in 2004 and 93 kg N ha^{-1} at the end of 2005 and 2006. Sewage sludge was applied superficially and the calculation of the required amounts was conducted according to the percentage of total N and dry matter contents (EPA, 1994) and taking into account the Spanish regulation (R.D 1310/1990) (BOE, 1990) regarding the heavy metal concentration for sewage sludge application. Tree heights were measured with a graduated ruler in October 2008 and pasture production was determined by taking four samples of pasture per plot at random (0.3 × 0.3 m^2) in May and December 2008. In the laboratory, the pasture samples were dried (72 hours at 60°C) and weighed to estimate dry matter production. Annual pasture production in 2008 was calculated by summing the consecutive harvests of the pasture production in that year. Data were analysed using ANOVA and differences between averages were shown by the LSD test, if ANOVA was significant. The statistical software package SAS (2001) was used for all analyses.

Results and discussion

In this study, tree height was lower in the MIN treatment than in the NF and PEL treatments ($P<0.001$) (Figure 1).
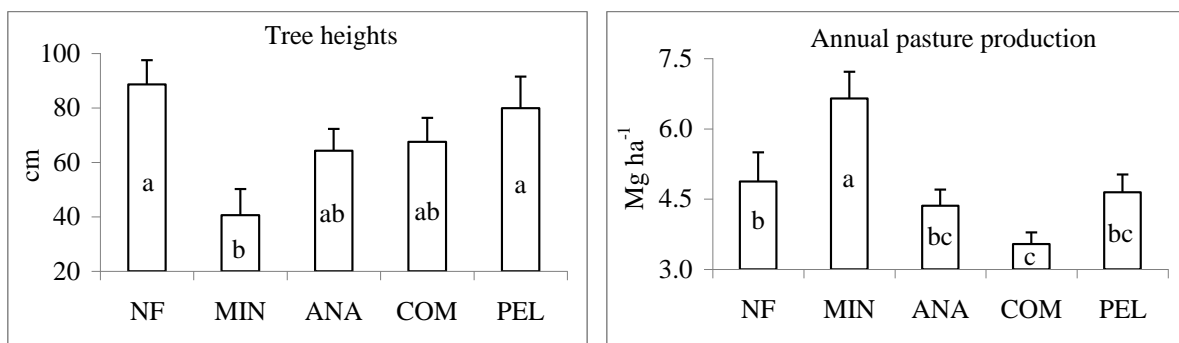
Figure 1. Tree heights (cm) (a) and annual pasture production (t [Mg] ha^{-1}) (b) under the different fertilizer treatments in 2008. NF: no fertilization, MIN: mineral; ANA: anaerobic sludge; COM: composted sludge and PEL: pelletized sludge. Different letters indicate significant differences between treatments. Vertical lines indicate mean standard error.

However, annual pasture production was increased with mineral fertilization (MIN) compared with the other treatments (NF, ANA, COM and PEL) ($P<0.05$). These results demonstrate a clear competition between pasture and trees in the MIN treatment, probably due to the intermediate soil pH (water soil pH: 5.6) which did not limit pasture development and, therefore, allowed a positive response of pasture to the mineral fertilization which consequently reduced the tree growth. Moreover, in this experiment, the competition generated between pasture and trees was high because the trees were in the first development stages and, therefore, their roots occupied the same soil depth as that of the pasture roots. Several studies have described that different root depths increase the sustainability and efficiency of use fertilizers (Nair and Kalmbacher, 2005), which used to be lower than 40% in agronomic soils (Jarvis and Menzi, 2004). Other authors, such as Rigueiro Rodríguez *et al.* (2000) and Mosquera Losada *et al.* (2006) also observed similar results to those found in our study in the first years after the establishment of silvopastoral systems in agronomic soils with *Pinus radiata* D. Don.
On the other hand, the positive effect of fertilization with pelletized sludge on tree height was previously observed by Rigueiro-Rodríguez *et al.* (2010) in silvopastoral systems established

under *Fraxinus excelsior* L. and could be explained because this type of sewage sludge was applied several times, thus facilitating the incorporation of sludge to soil and subsequent extraction of nutrients by trees. In general, organic fertilizers are characterized by their slow release of nutrients gradually over time due to their low mineralization rate, which is very important for trees as they are able to make better use of nutrients slowly released than pasture. Finally, it should be noted that PEL, besides increasing the tree height compared with MIN treatment, also reduced the application and storage costs compared with the ANA and COM due to its lower proportion of water, and therefore the use of this type of PEL sludge as fertilizer should be recommended.

Conclusion

Mineral fertilization increased annual pasture production and reduced tree heights due to the enhancement of the negative competition by nutrients generated between pasture and trees. However, the application of pelletized sewage sludge, split several times, implied an increase of the tree heights.

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Improved light availability of legumes in moderately N-fertilized mixed swards

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Abstract

A field experiment with five grassland species was carried out according to a simplex design in southern Sweden. The species represented the functional groups grasses, legumes and forbs, and were grown in two mixture types where but one species differed between them. Light transmission through the canopy was measured before each of four harvests. The harvested material was sorted into species, ground and analysed for $\delta^{13}C$. The $\delta^{13}C$ signature of all species but one was positively correlated with light transmission. There was also a positive correlation between the $\delta^{13}C$ signature of all species except one and the grass proportion of the plant community. The results indicate that mixing species with different leaf morphologies improves light availability and assimilation of legumes in particular.

Keywords: $\delta^{13}C$ signatures, forb, grass, legume, light transmission

Introduction

Empirical evidence indicates a positive relationship between grassland diversity and yield in both extensively and intensively managed systems (e.g., Finn *et al.*, 2013). One possible reason for this is complementary use of available resources among species, such as resource partitioning by legumes and non-legumes with respect to N acquisition (e.g., Frankow-Lindberg and Dahlin, 2013). Other complementarities may involve differences in the spatial arrangement of leaves between species in mixed plant communities (Anten and Hirose, 1999). Aboveground complementarities in light interception have so far received less attention than have belowground complementarities. The few relevant studies have been performed in extensively managed grasslands, where potentially tall-growing species have been able to fully exhibit their growth potential (e.g. Jumpponen *et al.*, 2005). Plant $\delta^{13}C$ signatures (i.e., the ratio of the stable isotopes of carbon, ^{12}C, and ^{13}C in plant leaves) are affected by environmental conditions such as light availability (i.e., more depleted $\delta^{13}C$ signatures occur with poor light availability). The aim of the study was to evaluate the effect of functional group composition on the $\delta^{13}C$ signatures of the species grown.

Materials and methods

A field experiment was established by drilling at Svalöv, Sweden (55° 55' N 13° 07' E, 55 m a.s.l.), in June 2007. The climate is cold–temperate with an annual mean temperature of 7.7 °C and annual mean precipitation of 700 mm. Twenty-two mixtures of *L. perenne*, *P. pratense*, *T. pratense,* and *C. intybus (Mixture type 1)*, ten mixtures of *L. perenne*, *P. pratense*, *T. pratense* and *M. sativa (Mixture type 2),* four monocropped stands each of *L. perenne*, *P. pratense* and *T. pratense,* and two monocropped stands each of *C. intybus* and *M. sativa* were established according to a simplex design, as described in detail in Frankow-Lindberg and Dahlin (2013). All mixtures and monocropped stands were sown at two seeding densities, i.e., 100% (high density) and 50% (low density) of the recommended seeding rates used in official variety-testing in Sweden, corrected for actual germination rates. The seeding rates in the high-density monocropped stands were 14.2 (*P. pratense*), 28.3 (*L. perenne*), 26.1 (*T. pratense*), 8.4 (*C.*

intybus), and 26.3 (*M. sativa*) kg ha⁻¹. In total, 48 plots were arranged in a completely randomized design, with an individual plot size of 17 m². In the harvest years, 100 kg of N ha⁻¹ yr⁻¹ was applied in split dressings (i.e., 40 kg of N ha⁻¹ in early spring and 20 kg of N ha⁻¹ for each summer regrowth in 2009). The plots were harvested four times in 2009 (i.e., 20 May, 24 June, 29 July, and 2 Sept.). The light transmission through the canopy (i.e., percent of incoming light (PAR)) of each plot was recorded before each harvest using a LiCor Quantum sensor (1 m long, five readings per plot) connected to a Quantum meter (LI-189, LM 189; Li-Cor, Lincoln, NE). The sown fractions from all harvests were ground per species to pass through a 1 mm screen, sub-sampled by riffle splitting, ball milled, and finally analysed for ^{13}C abundance, i.e., δ^{13}C expressed relative to international standard V-PDB (Vienna PeeDee Belemnite) using a PDZ Europa ANCA-GSL interfaced to a PDZ Europa 20-20 isotope ratio spectrometer (Sercon Ltd., Cheshire, UK). Linear correlations were calculated: between individual species' δ^{13}C signatures as the dependent variable and (i) light transmission through the canopy and (ii) functional group proportions of the harvested biomass as the independent variables. These were performed as completely randomized repeated-measures analyses with variables for sown density and mixture type included as fixed factors. Interactions between the independent variables and the fixed factors and between the independent variables and harvest occasion were also included.

Results and discussion

Increasing light transmission through the canopy was positively correlated with the δ^{13}C signatures of all species ($P < 0.05$) except *C. intybus*. Furthermore, there was a significant positive correlation between the δ^{13}C signatures of all species except *C. intybus* and the grass proportion in the harvested biomass ($P < 0.05$; Fig. 1).
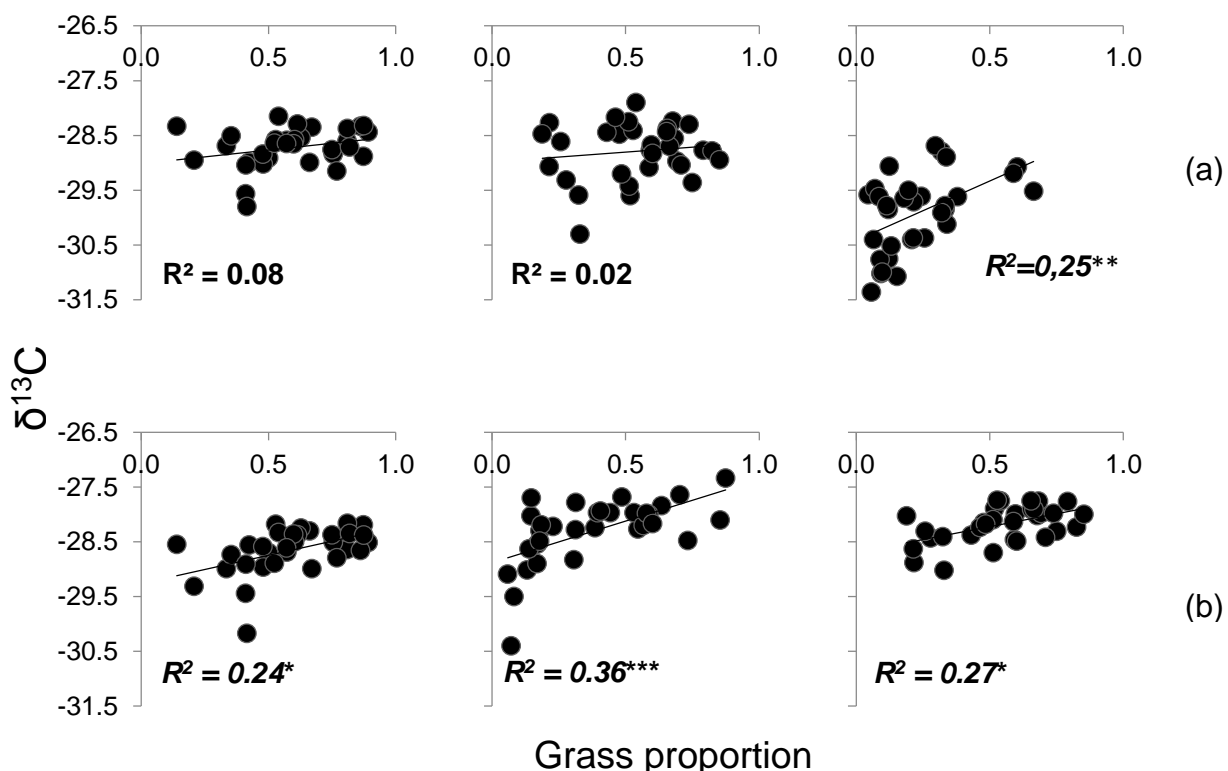
Figure 1. Correlations between grass proportion in the harvested biomass and species' shoot δ^{13}C signatures of a) perennial ryegrass and b) red clover in the first three harvests when light transmission was measured the day before each harvest occasion.

The positive correlation between $\delta^{13}C$ signatures and light transmission was strongest for the two legumes, which suggests that, despite height differences between them, their more horizontal leaf arrangement was a disadvantage in the competition for light. It is often noted that the N_2 fixation of legumes increases when they are grown in mixtures rather than monocropped stands, and this was also observed in the present experiment (Frankow-Lindberg and Dahlin, 2013). Part of this increase is likely due to the uptake of soil N by non-legume species, forcing legumes to increase N_2 fixation (Nyfeler *et al*., 2011), but the improvement in light conditions for legumes in mixtures with grasses may also make more energy available for this energy-demanding process.

Conclusion

In conclusion, our results suggest that mixing species of contrasting leaf morphologies and biomass distribution contributed to (i) increased light uptake by mixtures over monocropped non-legumes and (ii) better light availability for legumes in mixtures than monocultures.

Acknowledgements

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Nitrogen application strategies to mixed grass-legume leys

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Abstract

A field experiment was established in 2011 with the aim to evaluate different N application strategies on yield of mixed grass-clover leys. The treatments were 0, 40, 90, 160 or 250 kg N ha^{-1}, applied in single or split applications. The ley crop was harvested three times yearly in 2012 and 2013, and DM yield, botanical composition and digestibility were determined. The response to N application declined from the first to the second harvest year, despite a slight overall decrease in clover content. The economic return of N applications to the last harvest each year was negative for all treatments.

Keywords: grass-clover, red clover, white clover, N response

Introduction

Legume-rich leys can yield approximately the same as all-grass leys fertilized with 200 kg nitrogen (N) ha^{-1} (Kornher, 1982). However, even though grass-legume leys are the most commonly sown leys in Sweden most farmers apply N either in the form of slurry and/or mineral N fertilizers. This boosts yield and contributes to a more balanced botanical composition. Todays' seeding mixtures include more species than in the past, and there is a lack of Swedish data on how different N strategies affect yield, N response and digestibility of the typical legume-rich leys sown today. Therefore a field experiment was established to provide such data.

Materials and methods

A field experiment was established at Färjestaden, Sweden (56°38 N, 16°28 E) on 29 April 2011. The climate is cold-temperate with an annual mean temperature of 6.9°C and an annual precipitation of 500 mm. The soil at the site was a sandy soil with a pH of 6.7 containing 2.7% organic matter and soluble phosphorus and potassium were 20.4 and 6.9 mg 100g[-1] soil, respectively. The experimental plots received 19 kg of P and 199 kg of K in 2012, and 121 kg of K and 22 kg sulphur in 2013. The plots were undersown in spring barley which was harvested on 11 August 2011. The seeding mixture contained 14% *Trifolium pratense* (cvs. Nancy and Rajah), 5% *T. repens* (cv. Klondike), 38% *Phleum pratense* (cv. Lischka), 9% *Lolium perenne* (cvs. Kentaur and Foxtrot), and 34% *Festulolium* hybrid (cv. Hykor). The total seeding rate was 22 kg ha^{-1}. The plots were harvested three times in 2012 (29 May, 9 July, 27 August) and 2013 (6 June, 16 July, 26 August). Samples for the determination of dry matter, botanical composition and digestibility were taken at all harvest occasions. The experimental treatments were different N-application strategies. Here we report data from five of the treatments, namely: (i) no N application, (ii) 40 or (iii) 90 kg N ha^{-1} in spring and thereafter no N applications, (iv) 90 kg N ha^{-1} in spring + 35 kg N ha^{-1} to each regrowth, and (v) 120 kg N ha^{-1} in spring + 65 kg N ha^{-1} to each regrowth.

Results and discussion

There was no loss of plants despite the very long and cold winter in 2012-2013. Thus, total yield was high in both harvest years and averaged 12.5 tons and 13.2 tons ha^{-1} in 2012 and 2013, respectively. Approximately 50% of the yield was harvested at the first harvest occasion.
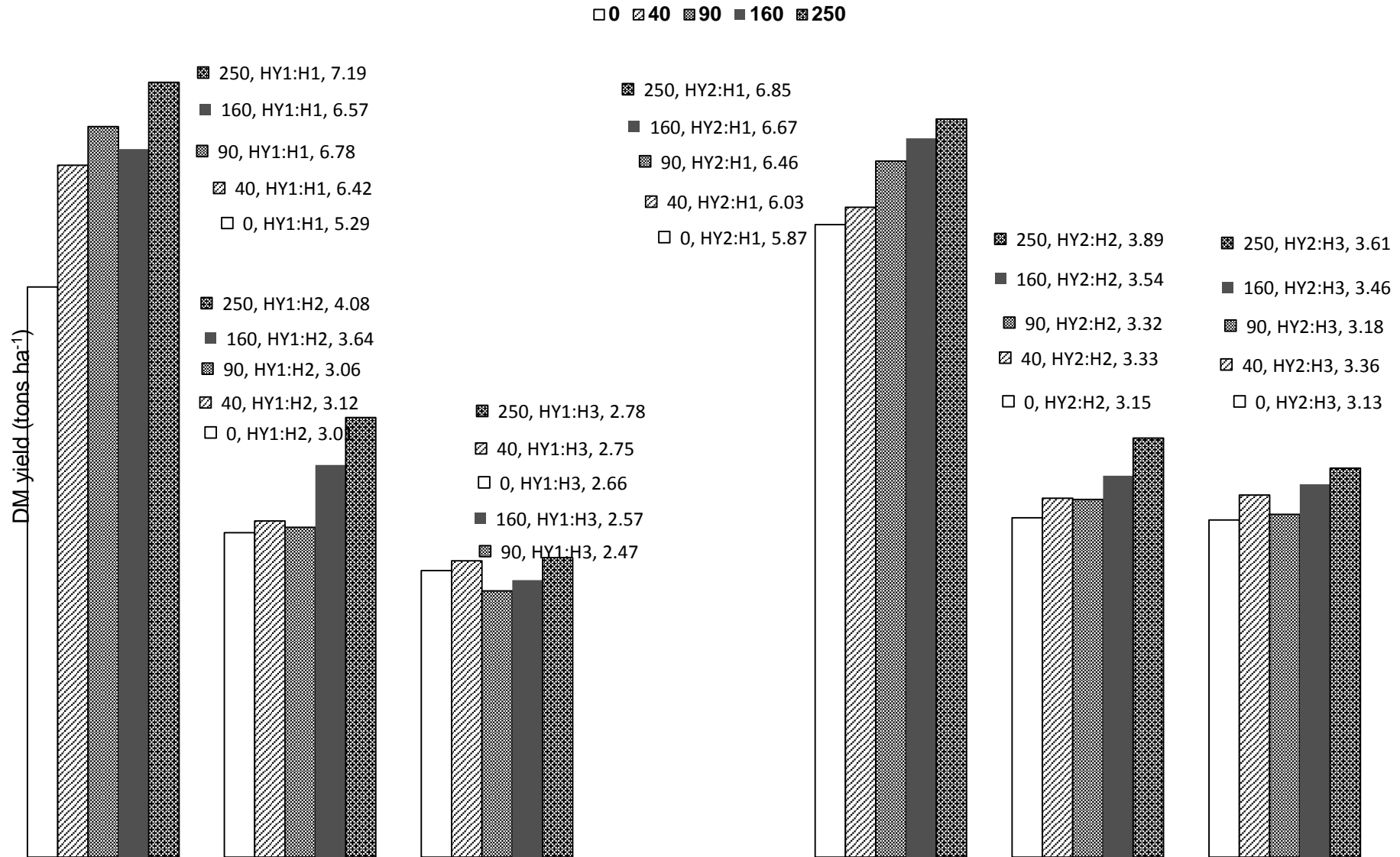
Figure 1. The effect of the amount of N applied on the yield of DM in each harvest year (mean ± LSD) and at harvest occasion. HY1 and HY2 refer to the first and the second harvest years, respectively, and H1, H2 and H3 refer to the first, second and third harvest occasion, respectively.

There were significant yield differences among treatments in the two first harvests in the first harvest year ($P<0.001$). The result was similar in the second harvest year, but with smaller differences among treatments. Applying N to the regrowths resulted in a significant yield increase ($P<0.001$) compared to the treatments that did not receive any N in the second harvest both years. In the third harvest, there was no significant difference among treatments in the first harvest year, and small, but significant, differences among some treatments in the second harvest year.

The clover content in the harvested DM was high in both harvest years, even though it declined somewhat from the first to the second harvest year (Table 1). This was mainly due to the content of red clover, which declined somewhat over the second harvest year, while that of white clover increased in all treatments (data not shown), mainly in the treatments that did not receive any N application to the summer regrowths. The drop in clover content over time, however, was largest at the highest N applications rates, which might be the reason for the increase in DM yield response to N in the last cut 2013 in these treatments.

Table 1. Dry matter yield response to nitrogen (kg DM kg N^{-1}) and clover content at each harvest occasion (% of dry matter)

	2012						2013					
	kg DM kg N^{-1}			Clover, % of DM			kg DM kg N^{-1}			Clover, % of DM		
	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3
0+0+0	-	-	-	41	59	74	-	-	-	38	53	61
40+0+0	14.2	-	-	33	57	76	8.9	-	-	35	56	58
90+0+0	28.3	-	-	19	42	66	4.0	-	-	23	44	57
90+35+35	16.6	18.0	-2.5	25	31	43	6.6	11.1	9.4	19	31	32
120+65+65	15.8	16.5	1.8	22	17	32	8.2	11.4	7.4	17	34	23

The DM yield response to N application was larger in the first harvest year than in the second harvest year (Table 1), despite similar or lower clover contents in the second harvest year compared to the first harvest year. The N response was similar at the two first harvest occasions, and poorer in the last summer regrowth, in the treatments that received N to the regrowths. This can be explained by the decrease in grass contribution over the season in all treatments.

The overall response to N application was 11.4 and 9.5 (160 kg N ha^{-1}), and 12.4 and 8.8 (250 kg N ha^{-1}) kg DM kg N^{-1}, in the first and the second harvest years, respectively. This is a slight increase in relation to the responses obtained with seeding mixtures in the past, based on *T. pratense, P. pratense* and *Festuca pratensis* (Kornher, 1982).

The *in vitro* digestibility of the crop was unaffected by the treatments in all harvests except the last harvest in the first harvest year, when the treatment receiving 250 kg N ha^{-1} had the highest digestibility ($P<0.001$).

Assuming a value of the harvest of 0.14 € per kg DM, an N cost of 0.89 € per kg, and a spreading cost of 17.78 € per occasion, we find that the application of N was economic for the first two harvests in 2012. This was not the case in 2013, when the spring application of N was uneconomic to all treatments except the treatment with the highest application rate, and then barely so. However, the applications to the second harvest in 2013 yielded a positive economic return. In neither year was it economic to apply N to the last summer regrowth.

Conclusion

The response to N applications was strongest in the first harvest year. Applying high N rates the first harvest year resulted in a decline in clover content in the second harvest year. In neither harvest year was it economical to apply N to the last harvest.

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Potential of short-term nitrogen transfer between *Trifolium repens* and the grasses *Festuca* gr. *rubra* and *Brachypodium pinnatum* in highland grasslands

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Abstract

This research was undertaken to determine the occurrence of short-term N transfer between the legume *Trifolium repens* and grasses *Festuca* gr. *rubra* and *Brachypodium pinnatum*. In Europe, *F. rubra* is always associated with species-rich grasslands whereas *B. pinnatum* may constitute, under particular conditions, monospecific covers. The hypothesis underlying this experiment is that species that are more reliant on short-term N transfer from legumes might be more prone to constitute multispecific covers (and *vice-versa*). Undisturbed blocks of vegetation and soil were collected in highland grasslands and transplanted to a greenhouse, where a ^{15}N leaf-feeding experiment was applied to study the fate of the labelling. One month later, a high percentage of the ^{15}N applied was recovered in soils (up to 25%, on average) but, despite the ^{15}N exuded in the soil, neither *F. rubra* nor *B. pinnatum* were significantly ^{15}N enriched. The high C/N of these soils and the occurrence of some outliers suggest that this research merits more study.

Keywords: N acquisition, legume-grass interaction, semi-natural grassland, species-rich cover.

Introduction

In highland grasslands over Europe, grasses are the dominant taxa. In floristic inventories made in species-rich grasslands their occurrence often exceeds 90% of the total cover. The grasses constitute a diverse group of species that mostly share endemicity and adaptation to a harsh climate and frequent soil-nutrient constraints. The relationship that these dominant grasses establish with the rest of the species influences the final floristic diversity and community functionality. In grasslands of the Pyrenees, under given conditions of change, some grass species that belong to species-rich communities, such as *Nardus stricta* and *Brachypodium pinnatum,* may increase and displace other species, degrading the community. The process has also been reported in other European grasslands. The dominance of *N. stricta* has been associated with decreasing herbivory and with selective grazing by sheep (Sebastià *et al.*, 2008) and the constitution of monospecific covers of *B. pinnatum* is linked to prescribed burnings and to low grazing pressure, among other factors (Canals *et al*., 2014). Both species develop a powerful rooting system and a dense turf, and have a rapid decline of digestibility, which make them less attractive to livestock. Although these traits may help to explain its potential to displace other species in the community, there are other successful, competitive and productive grasses, such as *Festuca* gr. *rubra* and *Agrostis capillaris* that, even being dominant and in conditions of poor grazing, do not cause a loss of community richness and diversity.

Different reasons may help to explain what makes a given species more prone than others to displace other taxa in the community (or in contrast, what makes it more prone to coexistence). One hypothesis is that some species may be more reliant than others on neighbouring species, i.e., facilitation is relevant for species development. The assumption is that the species needs diversity or, at least, the occurrence of some given taxa (such as legumes), to develop successfully. Facilitation mechanisms are known to be very important in constrained environments, as those of highlands. The availability of critical nutrients for plant growth, such as nitrogen (N), is limited, since mineralization is restricted and microbiota compete efficiently for N (Jaeger *et al.*, 1999). Plants have developed different strategies for an efficient N-

acquisition in this situation. In this research we are interested in assessing whether a short-term N transfer (Figure 1) occurs between the legume *Trifolium repens* and the grasses *B. pinnatum* and *F. rubra.*

Short-term N adquisition possibilities (*Motorway N transfer*)

Direct

Indirect (through soil)

Legume-Mycorrhiza-Grass   Legume-Mycorrhiza-Soil-(Mycorrhiza)-Grass   Legume Exudates-Soil-(Mycorrhiza)-Grass
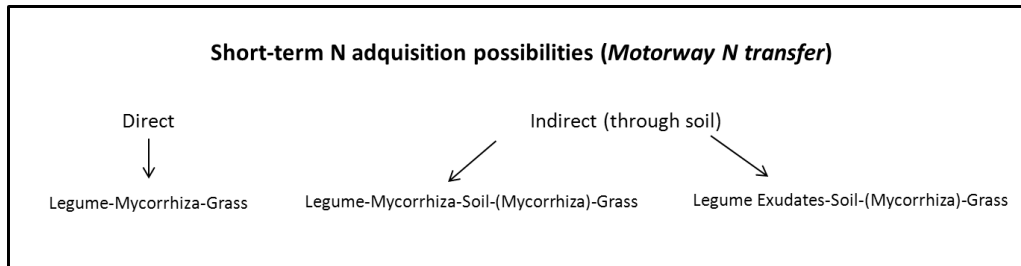
Figure 1. Conceptual model of short-term N transfer pathways between a legume and a grass.

Methods

In early March 2012, thirty 20×20×20 cm undisturbed blocks of soil and vegetation containing the three species of the study were collected in a semi-natural grassland of the south-western Pyrenees (1100 m a.s.l.; 43°0/N, 1°10/W). The blocks were transported to UPNA greenhouse facilities and placed intact in rectangular pots that had a bottom layer of sand and turve. Plant development was ensured over the following months by regular watering with distilled water. On 24 May 2012 the isotopic ^{15}N solution was applied to *T. repens* plants as described by Marty *et al.* (2009). Grasses were cut to 1-cm of the soil the day before to reduce ^{15}N dilution, and 20-40 *T. repens* leaflets per pot were labelled by placing 1µL of (^{15}NH$_4$)$_2$SO$_4$ 50mM (at ^{15}N 99 atom. %) in two wounds of 2 mm^2 at the abaxial face of leaflets. Nineteen pots were labelled and two pots were used as controls for ^{15}N natural abundance (9 pots were discarded because of the disappearance of the legume or the two grasses). Thirty days after the labelling, plants were removed from the soil and separated into species and tissues (green aboveground and roots). Plant samples were rinsed three times with distilled water and dried at 65°C for 48h for dry weight determinations. A composite sample of soils was obtained per pot, which was dried at 100°C till constant weight. Then, plant and soil samples were finely ground and sent to SIRF-UC Davis for isotopic analyses with a mass spectrometer (PDZ Europa ANCA-GSL, Sercon Ltd). Dependent variables considered in the statistical analyses were % N total, ^{15}N enrichment (ug^{15}N/g) and % ^{15}N recovered:

$\mu g\ ^{15}N/g = [\%\ N\ total\ \text{x}\ (sample\ ^{15}N - natural\ ^{15}N)]\ x\ 100$
$\%\ ^{15}N\ recover = [(\mu g^{15}N\ recovered/pot)/(\mu g^{15}N\ initially\ applied)/pot]\ x\ 100$

Data on % total N were analysed using a parametric split-plot design (species was the main-plot, tissue the subplot and pot the replicate). Plant ^{15}N enrichment and % ^{15}N recovered were analysed using a T test, which compared whether the average of enrichment/recovery differed significantly from zero. ^{15}N enrichment of *F. rubra* leaves could not be transformed successfully and was analysed by the Wilcoxon rank procedure. Software used was SPSS Statistics and Statistix v.8.

Results and discussion

Total N in plants differed significantly between single treatments (species F=74.53, *P*<0.0001; tissues F= 212.86; *P*<0.001) and their interaction (F=22.53, *P*<0.001). As expected, the legume displayed the highest % N in plants. However, when considering tissues separately, aerial parts of *T. repens* and *F. rubra* had similar % total N, higher than those in *B. pinnatum*, whereas legume roots concentrated more N than roots of grasses.
Regarding the ^{15}N enrichment, values were different from zero for *T. repens* (t_{root}=13.741 *P*<0.001, $t_{aboveground}$=6.667 *P*<0.001) and for soil (t_{soil}=3.747 *P*<0.001). A major recovery of

[15]N occurred in soils, 24.8%±6.27 (average ± std error), followed by *T. repens* 11.91%±3.01, which indicated that the enriched solution penetrated within the plant and was exudated to the soil in a high percentage (almost 1/4 in the soil after one month). Despite ^{15}N exudation, neither *F. rubra* nor *B. pinnatum* tissues displayed a significant ^{15}N enrichment (*F. rubra* $t_{root}=0.16$ $P=0.869$, Wilcoxon test$_{aboveground}$ $P=0.841$; *B. pinnatum* $t_{root}=-1.148$ $P=0.272$, $t_{aboveground}=0.266$ $P=0.795$) (Figure 2). These results contrast with those of Marty (2009) who found higher ^{15}N enrichments in *F. eskia* than in *N. stricta* in a comparable experiment. On the one hand, the high C/N ratio of our soil, around 15, suggests a rapid use of released N and a strong competition with microbes, which may outcompete grasses for the use of N exudates (Jaeger, 1999). On the other hand, the occurrence of an outlier in pot 20, where both *T. repens* and *F. rubra* exhibit the highest ^{15}N enrichment (*T. repens*: 44.44 µg[15]N/g aboveground and 9.99 µg[15]N/g belowground, and *F. rubra*: 2.43 µg[15]N/g aboveground), leaves open the possibility of N transfer between both species, which deserves more study.
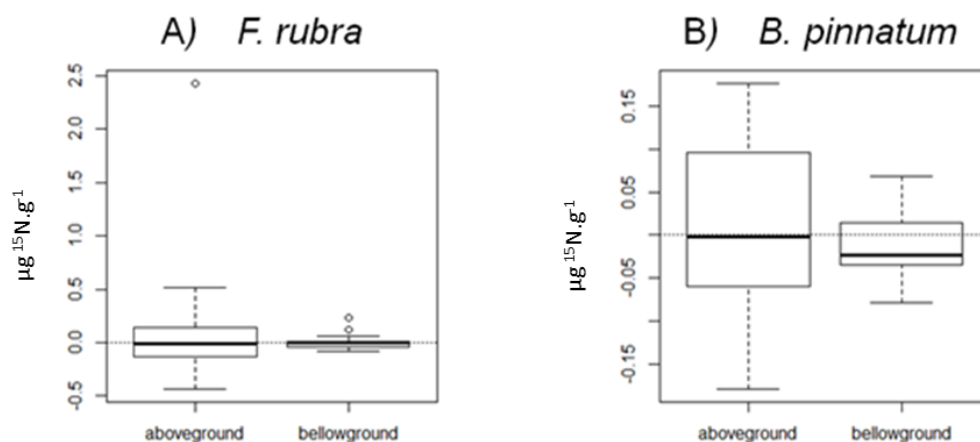
Figure 2. A) Excess of [15]N (µg/g) in aboveground and belowground tissues of *F. rubra.* B) Excess of [15]N (µg/g) in aboveground and belowground tissues of *B. pinnatum.*

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Root architecture of interspecific hybrids between *Trifolium repens* L. and *Trifolium ambiguum* M. Bieb. and their potential to deliver ecosystem services

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Abstract

The potential of grasslands to deliver ecosystem services and mitigate some of the impacts of climate change is increasingly being recognized. Backcross hybrids between the stoloniferous *Trifolium repens* L., and the related rhizomatous species *T. ambiguum* M. Bieb have been produced using *T. repens* as the recurrent parent. The differences between parental species and the backcrosses in root morphology were studied in 1-m long pipes. The parental species differed in root distribution and in root weight distribution, with root weight of *T. ambiguum* significantly greater than *T. repens* in the 0.1 m to 0.5 m root zone. The backcrosses exhibited root characteristics intermediate between the parents. The extent to which such differences in root architecture may influence soil structure and deliver ecosystem services is discussed.

Keywords: *Trifolium repens*, interspecific hybrids, moisture stress, root distribution, ecosystem services.

Introduction

Adaptation of agriculture to predicted climate change scenarios is increasingly recognized as important for UK grassland production. Increasing winter rainfall leading to flooding and greater incidence of summer droughts are likely to be more common (Macleod *et al*., 2013). Breeding of improved forage varieties able to tolerate periods of variable rainfall and that can help alleviate the impacts of flooding is now a key objective of the IBERS forage breeding programmes. The most important forage legume component of temperate pastures is white clover (*Trifolium repens* L.), a nitrogen-fixing species that produces forage of high quality. Hybrids have been successfully developed between white clover and Caucasian clover (*T.ambiguum* M. Bieb) to introgress the rhizomatous trait from Caucasian clover into white clover (Marshall *et al*., 2001) as a strategy for improving tolerance of moisture stress. Comparison of the BC1 and BC2 hybrids with the parental species (Marshall *et al*., 2001) showed that the backcross hybrids maintained a higher leaf relative-water content (RWC) than white clover at comparable levels of soil moisture. The basis of this improved drought tolerance is unclear although preliminary studies suggest that the hybrids have a higher proportion of their DM yield in roots than *T. repens* (Marshall *et al*., 2001). The objective of this study was to analyse the root distribution of the parental species and backcross hybrids.

Materials and methods

Hybrid development and their morphological characterization were described previously (Marshall *et al*., 2001). Four genotypes of the *T. repens*, *T. ambiguum*, BC1 and BC2 hybrids were cloned to provide 6 plants of each genotype. Clonal plants of the parental species and hybrids were transplanted into plastic pipes (1 m deep × 15 cm diameter) with several drainage holes in the base into which was inserted a polythene tube filled with soil. The pipes were placed on a gravel bed within a glasshouse maintained at ambient temperature. After 10 weeks the polythene tube was removed from the pipe and the aboveground foliage cut with hand-held shears and the root column separated into 10-cm deep longitudinal sections. The roots within each of these sections were carefully removed by gently washing each section under running

water. The dry weight of the roots within each 10 cm section was then determined after drying at 80ºC for 24 hours in a forced draught oven. The experiment was established as a split-plot design with four replicate blocks, species as whole plots and genotypes as sub-plots.

Results and discussion

Within the relatively limited objectives of this experiment, the parental species *T. repens* and *T. ambiguum* differed in the distribution of roots within the soil profile and in root weight distribution. There was a significant difference between species in root dry weight up to depths of 0.5 m, and also significant differences between genotypes within species (Table 1). However, at depths below 0.5 m, differences between species were small and insignificant and are not shown.

Table 1. Significance levels for effect of species and genotype within species on root dry weight at different depths

	Root depth (m)				
	0.1	0.2	0.3	0.4	0.5
Species	*	**	***	***	***
Genotypes within species	***	**	**	**	NS

NS, not significant; *$P<0.05$; ** $P<0.01$; ***$P<0.001$

Root dry weights of *T. repens* and *T. ambiguum* in the 0 to 0.1m root zone were comparable (Figure 1); however, in the subsequent 0.1 m zones, to a depth of 0.5 m, the root dry weight of *T. ambiguum* was significantly greater than that of *T. repens* (Figure 1).
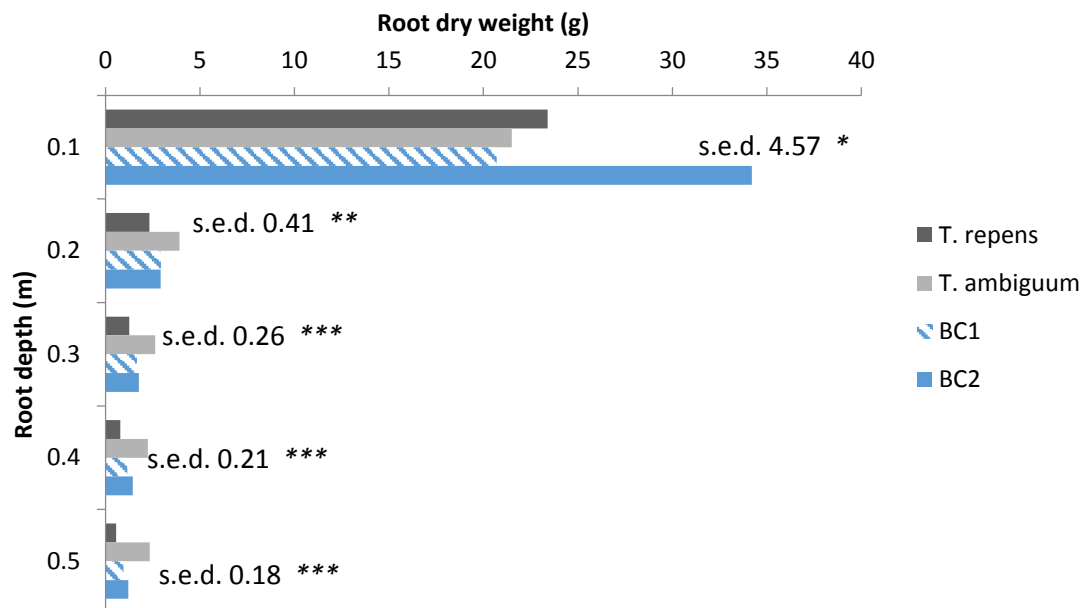
Figure 1. Root dry weight in 0.1 m sections of soil columns containing *T. repens*, *T. ambiguum*, BC1 and BC2 hybrids. NS, not significant; *$P<0.05$; ** $P<0.01$; ***$P<0.001$

Previous studies have shown that *T. ambiguum* has a higher proportion of dry matter in roots than *T. repens* and that these differences may be one factor that improves tolerance of soil moisture deficit. Apart from the 0 to 0.1 m root zone, where the BC2 had the greatest root dry weight, the root dry weight of the BC1 and BC2 hybrids were not significantly different and were generally intermediate between the two parental species.

Significant differences in root dry weight were found between genotypes of *T. repens* and of the BC1 and BC2 hybrids up to a depth of 0.4 m but not between genotypes of *T. ambiguum*. This suggests there is extensive variation in root density between genotypes of *T. repens* and,

not surprisingly, this variation is also observed within the BC1 and BC2 hybrids. Further work is clearly needed to explore this within other backcross families and to study whether comparable results are replicated in field conditions and when in mixed swards with companion grasses. Recent studies have shown that grass species differ in root distribution (Macleod *et al.*, 2013), and that such differences can contribute to improved soil porosity with improved water infiltration, delivering valuable ecosystem services particularly in alleviating the impact of flooding. This present study suggests there may be potential to exploit differences in root architecture within these clover hybrids to develop white clover varieties that will also deliver ecosystem services. Experiments to validate the impact of these new varieties in mixed swards will be the focus of future studies.

Conclusions

The parental species differed in root distribution and in root weight distribution, with root weight of *T. ambiguum* significantly greater than *T. repens* in the 0.1 m to 0.5 m root zone with the backcross hybrids exhibiting root characteristics intermediate between the parental species. Difference in root distribution is a factor influencing the extent to which plants are able to tolerate moisture stress but it may also have beneficial effects on soil porosity delivering ecosystem services.

Acknowledgements

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Interactive N supply and cutting intensity effect on canopy height at 95% light interception

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Abstract

The aim of this study was to evaluate the impact of pasture management practices on leaf canopy height (LCH) at 95% light interception (LI), since this is a valuable strategy of defoliation frequency. These relationships were investigated over two years with monocultures grown in a fully factorial block design crossing six C_4 pasture grass species, two cutting intensities and two N levels. We found variations on LCH at 95% LI, mainly across seasons, but also between treatments. The range of these variations was species-dependent. Therefore, in order to maintain a target IL level, grassland managers should cut or graze at different heights according to the species and seasons. N fertilization and an increase in cutting height above ground level can provide a sward structure that allows lower pre-cutting/grazing height and, consequently, shorter intervals between defoliations.

Keywords: C_4 grasses, defoliation frequency, response to management

Introduction

The limitations of adopting standard pre-defined rest periods to control rotational stocking strategies are well known, and highlight the importance of using a plant growth-based criterion to define intervals between successive grazings. This leads to the creation of new methods to manage the resources in regard of these aims than in terms of fixed rest periods, as is still prescribed in recommendations based on classic grazing systems. For instance, recent studies have adopted the criterion based on sward light interception, since it is effective for dealing with the variability of herbage accumulation throughout the year (e.g. with *Megathirsus maximum* cv. Mombaça, Da Silva *et al.*, 2009), particularly with C_4 grass pastures. As a result, ceiling yield with a better control of stem and dead material accumulation has been reached when 95% of light is intercepted by the canopy. Further, correlations between light interception (LI) and sward height have been performed in order to obtain an easy pasture management practice. A consistency seems to emerge of leaf canopy height (LCH) at 95% LI throughout the year, regardless of swards being vegetative or reproductive. However, variations on LCH were related with Tifton 85 (Silva *et al.*, 2013) due post-grazing residues used. Therefore, in order to obtain a better understanding of these relationships, an experiment was set up with a broader range of C_4 grass pastures, since these canopies displayed a great vertical heterogeneity, particularly when nutrients are limited. This paper compares the impact of management practices (cutting intensity and N supply) on the LCH of six C_4 grass species at 95% LI. In order to do this, the species studied were grown in monocultures, and a simulated rotational defoliation by mechanical cutting was adopted in order to control more easily the extent of the plant parts removed as well as the height at the targeted IL.

Materials and methods

The study was based on six perennial C$_4$ grasses (*Axonopus catharinensis* (Ac), *Cynodon spp.* hybrid Tifton 85 (Cs), *Hemarthria altissima* cv. Florida (Ha), *Megathirsus maximus* cv. Aruana (Mm), *Paspalum notatum* cv. Pensacola (Pn) and *Urochloa brizantha* cv. Marandu (Ub)) that are widely used in Brazilian livestock. The experimental site was located at the Agronomic Institute of Paraná, Ponta Grossa-PR (25° 07' 22'' S 50° 03' 01'' W). The species were planted in pure stands in 2010 (4.5 m²). The cutting intensity and N fertilizer treatments (zero vs. 300 kg N ha^{-1}year^{-1}) were started in January 2011. Plots were cut when the LI of swards were 95%. At this moment, the sward height was measured (10 measures per plot, only in leaves and using a sward stick), and the residual kept was 30% (C30, i.e. high cutting intensity) and 50% (C50, i.e. lower cutting intensity) of this LCH. LI was monitored regularly with a ceptometer (Decagon LP-80 AccuPAR) placed at ground level and above the grass canopy. Data collected over two consecutive years (from spring to fall 2011-2012 and 2012-2013) were evaluated. An analysis of variance was performed on data of height at 95% of canopy LI using R software (R Development Core Team, 2013) to test statistical significance of main factors: year, block, season (nested in each year), species, cutting intensity, N supply and their interactions (except with block). This analysis was performed using GLM model, assuming block to be a random effect and the others as the fixed effects. Prior to ANOVA, height was transformed by log to normalize the data.

Results and discussion

Outputs of the ANOVA for height at 95% of canopy LI are shown in Table 1.

Table 1. Percentage of variance explained (VE) and statistical significance from the analysis of variance for height at 95% of canopy light interception. Df, degrees of freedom.

	Df	VE	*P*
Year	1	4.2	***
Species	5	60	***
Block	2	-	ns
Cutting intensity (CI)	1	1.5	***
N supply	1	<1	**
Season(Year)	2	7.2	***
Season(Year)×species	10	3.1	***
Species×N	5	<1	*
Species×CI	5	<1	**
Season(Year)×N	5	<1	*
Season(Year)×CI	5	<1	*
Season(Year)×Year	2	1.2	***
Season(Year)×Year×Species	15	1.5	***
Season(Year)×N×Species	9	<1	**

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. Only significant interactions for at least one variable are presented.

Species was the large source of variation, explaining 60% of total variance, with *M. maximus* having the greatest height, while *U. brizantha* displayed the lowest value for this variable (Table 2). Since these swards have a wide range of plant morphology and structure, varying from prostrate (e.g. Ac) to tall tussock, erect growing plants (e.g. Mm), a specific height at this target IL level (i.e. 95%) can be expected. Year (37±0.78 and 42±0.86 cm, on average, for the first and second year, respectively) and mainly season had also important effect on the variable studied (Table 1, and see Table 2 for means). In addition, season × species interaction was the most important in terms of variance explained (Table 1). Hence, means per species within each season are shown in Table 2. A considerable variability was observed over growing seasons, except for *U. brizantha* and *A. catharinensis* (Table 2).

Table 2. Seasonal means for leaf canopy height (cm) at 95% of light interception for each species. Data are means (± s.e.) of two labels of nitrogen supply, two cutting intensities, three blocks and two years. Data show the season × species interaction.

Species	Spring	Summer	Fall	Means
Axonopus catharinensis	35 ± 1.34 ab	38 ± 0.97 a	33 ± 1.95 b	36 ± 0.71 CD
Urochloa brizantha	27 ± 0.76 a	23 ± 0.64 b	22 ± 0.91 b	24 ± 0.47 E
Hemarthria altissima	42 ± 1.90 b	53 ± 1.69 a	40 ± 1.82 b	46 ± 1.20 B
Megathirsus maximus	62 ± 2.02 a	58 ± 1.60 b	45 ± 2.37 c	56 ± 1.26 A
Paspalum notatum	34 ± 2.36 b	40 ± 0.82 a	32 ± 1.95 b	37 ± 0.87 C
Cynodon spp.	35 ± 1.80 b	39 ± 0.81 a	28 ± 1.73 c	35 ± 0.84 D
Means	41 ± 1.26 A	42 ±0.84 A	33 ± 0.92 B	-

Values followed by the same letters within a line (uppercase letters for means) do not differ significantly.

The majority of species had a higher height in summer with lower values in fall (Table 2). A reason for the increase in height in some seasons would probably have been caused by stem formation due plant maturity developmental stage. Therefore, in order to maintain 95% as a target IL level, grassland managers should cut or graze at different height for reproductive or vegetative growth according to the species. This generates variation in the management of the system between seasons of the year, but need careful control if sward state that optimizes herbage production is to be achieved effectively. There were species × N and species × cut intensity interactions for height at cutting date (Table 1). Fertilizer N significantly decreased the height at the 95% LI for *M. maximus* (- 7 cm) and *A. catharinensis* (-3 cm). An increase in cutting intensity (or decrease in residual height) was associated to an increase in the height at cutting date, mainly for *M. maximus* (+ 8 cm), *U. brizantha* (+ 4 cm), *H. altissima* (+ 5 cm) and Tifton 85 (+ 3 cm). Only *P. notatum* was not affected by both treatments. Our findings reveal that N fertilization and an increase in cutting height above ground levels help to reduce the leaf canopy height pre-cutting. This result can be attributed to a probably maintenance of the sward structure leafy and with a higher tiller density (Silva *et al*., 2013), which allows the canopy to intercept 95% of the incident light faster, i.e. with a lower height.

In summary, variations for LCH at 95% LI were observed between treatments and seasons, with a large range for this last factor. For instance, while variations on LCH across seasons can reach 11 cm for Tifton 85 (see Table 2), changes due the treatments were around 3 cm (C30 = 37 ± 1.36 cm *vs*. C50 = 34 ± 1.04 cm). For this reason, a narrow pre-cutting or grazing height range (e.g. 28 ± 3.0 cm during the fall for Tifton 85) could be suggested for each species and according to its phenological stage or growing period, regardless management practices. However, the level of herbage depletion and N fertilization are also powerful tools for managing and controlling sward structure in order to optimize herbage intake by herbivores.

Conclusions

The results of the present study indicate that variations on LCH at 95% LI occurs between treatments, but mainly across seasons. Furthermore, the range of these variations was species-dependent. Future studies could focus on the effect of these sward surface heights on ruminants' performance.

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Interactive N supply and cutting intensity effect on leaf nutritive value of C[4] grasses

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Abstract

Monocultures of six warm-season perennial grasses were compared in a factorial field design of two N levels (zero and 300 kg N ha^{-1}year^{-1}) and two cutting intensities (i.e., the residual kept was 30% and 50% of the canopy height at 95% light interception). The main components of plant nutritive value were measured in two successive growing periods. The species studied exhibited a significant range on leaf lamina quality and leaf proportion. Overall, changes on these parameters were greatest for N fertilizer than cutting-intensity treatments.

Keywords: light interception, nutritive value, response to management

Introduction

For forage crops, it is well established that the nutritive value is strongly affected by management factors through changes on the three components of plant quality, i.e. leaf and stem quality and leaf proportion (Duru *et al.*, 2008). For instance, with tropical/subtropical grass species, a higher nutritive value has been achieved when 95% of canopy light interception (LI) is used as a cutting frequency in rotational stocking (Da Silva and Carvalho, 2005). This response is largely a consequence of a higher leaf:stem ratio, since leaf quality is higher than those of stems. However, differences in quality among species may be attributed to differences in morphological features of their leaves (Pontes *et al.*, 2007), since these values can reflect the strategy of plant species whereby adaptation to variations in land use are achieved. Therefore, the leaf lamina itself plays an important role on plant species quality according to growing conditions, particularly in situations where swards are leafy. The aim of this paper was to examine the effect of cutting intensity and N fertilizer application on leaf nutritive value and leaf proportion of six perennial C[4] grasses, growing in monoculture. The 95% LI criterion was adopted as a cutting frequency. Knowing how these nutritional characteristics vary with management may aid decision-making in order to have more effective grazing programmes.

Materials and methods

The study was based on six perennial C[4] grasses (*Axonopus catharinensis* (Ac), *Cynodon spp.* hybrid Tifton 85 (Cs), *Hemarthria altissima* cv. Florida (Ha), *Megathirsus maximus* cv. Aruana (Mm), *Paspalum notatum* cv. Pensacola (Pn) and *Urochloa brizantha* cv. Marandu (Ub)) that are widely used in Brazilian livestock. The experimental site was located at the Agronomic Institute of Paraná, Ponta Grossa-PR (25° 07' 22" S 50° 03' 01" W). The species were planted in pure stands in 2010 (4.5 m²). The cutting intensity and N fertilizer treatments were started in January 2011. Plots were cut when the LI of swards were 95%. At this moment, the sward surface height was measured, and the residual kept was 30% (C30, i.e. high cutting intensity) and 50% (C50, i.e. lower cutting intensity) of this original canopy height. We simulated rotational defoliation by mechanical cutting in order to easily compare several species and control the extent of the plant parts removed. Further, two contrasting N levels (zero and 300 kg N ha^{-1}year^{-1}, N0 and N300, respectively) were compared. At the cutting date, laminas (youngest fully expanded lamina) were selected at random from vegetative tillers and cut off,

oven dried at 60 °C for 48h, and milled through a 1 mm screen. Our focus is concentrated at the leaf lamina level in order to have a more accurate prediction about the effect of the management practices on species quality, i.e. with no effect of plant components proportion. One sample per plot was collected over two consecutive growing periods (from spring to autumn 2011-2012 and 2012-2013). Each sample was analysed via near-infrared reflectance spectroscopy (NIRS) procedure (FOSS-NIRSystems 5000; CEPA laboratory, Passo Fundo-RS, Brazil) for crude protein (CP), dry-matter digestibility (DMD), neutral detergent fibre (NDF) and acid detergent fibre (ADF). Leaf proportion was measured from representative samples harvested above cutting height. Analyses of variance were performed using Statgraphics Centurion XV to test statistical significance of main factors: year, block, species, cutting intensity, N supply and their interactions (except with block). All response variables were analysed using GLM model, assuming year and block to be a random effect and the others as the fixed effects.

Results and discussion

Outputs of the ANOVA for nutritive value parameters are shown in Table 1.

Table 1. Percentage of variance explained and statistical significance from the analysis of variance for crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), dry-matter digestibility (DMD) and leaf proportion (LP).

	df	CP	NDF	ADF	DMD	LP
Growing period (GP)	1	ns	9.3*	ns	ns	ns
Block	2	ns	ns	ns	ns	ns
N	1	39***	16***	ns	ns	8.6***
Cutting intensity	1	1.6**	ns	ns	ns	1.5**
Species	5	27*	38*	50**	50***	42***
GP×Species	5	5.2***	6.8***	3.2*	3.2*	-
GP×N	1	-	-	1.5**	1.5**	-

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; df, degrees of freedom; ns, not significant. Only significant interactions for at least one variable are presented.

Species was a large source of variation for NDF, ADF, DMD and leaf proportion, and explained between 38 and 50% of total variance (Table 1). Means per species are shown in Table 2.

Table 2. Percentage (±standard error) on leaves of crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and dry-matter digestibility (DMD) for six perennial C_4 grasses. LP, leaf proportion (%).

Species	CP	NDF	ADF	DMD	LP
Axonopus catharinensis	13±0.54c	67±0.53b	34±0.68b	62±0.53b	60±2.35b
Cynodon spp.	16±0.55ab	74±0.80a	35±1.06b	61±0.82b	59±1.75bc
Hemarthria altissima	15±0.59abc	71±0.90ab	35±0.79b	62±0.62b	36±2.14d
Megathirsus maximus	17±0.66a	70±0.64ab	35±1.13b	61±0.88b	58±2.35bc
Paspalum notatum	13±0.46bc	74±0.54a	40±0.61a	58±0.47c	54±3.06bc
Urochloa brizantha	16±0.38a	68±0.81b	27±0.57c	68±0.44a	81±2.08a

Values followed by the same letters within a column do not differ significantly.

The highest values of leaf proportion and DMD were achieved for *U. brizantha* (Table 2). This species had also the lowest ADF (27%). At the opposite, *P. notatum* had the lowest DMD (58%) and the highest ADF (40%) values in leaves, and *H. altissima* displayed the lowest leaf proportion (36%). The CP percentage of all species ranged from 13 (Ac) to 17% (Mm), which is above the minimum CP level required for rumen function (7.5%, Van Soest, 1994). The NDF percentages recorded in this experiment ranged from 67 (Ac) to 74% (Cs and Pn), which were similar to those reported by Soares *et al.* (2009) for Ac, Cs, Ha, Mm and Ub, and where the NDF average in leaves was 70%. For CP, N supply effect was more important than species effects, accounting for 39% of total variance. This result confirms a previous report that the CP concentration of herbage varies mainly with the nitrogen status on the plant (Lemaire and

Gastal, 1997). For all species, N supply significantly increased the CP (+3.5%) and leaf proportion (+11%), and decreased the NDF (-3.2%) in leaves. The higher leaf proportion at N300 could be a consequence of a probably higher tiller density and an increase in leaf size. The CP percentage was significantly higher in C50 (15.5±2.89%) than in C30 (14.8±2.92%), probably due a higher opportunity in increasing the N resorption efficiency. Further, a higher leaf proportion was also observed in C50 (62±1.58%) when compared to C30 (57±1.73%), because increasing the level of depletion of the pre-cutting canopy height leads to a marked increase in stem and dead material (data not shown). The NDF was significantly higher in the first growing period (72±4.32%) than in the second (70±3.94%). In relationship to the interactions, the year×species was the most important in terms of variance explained. These results highlighted a sampling date effect despite lower differences on leaf quality over time than stems (Duru *et al.*, 2008). However, despite significant interactions between growing periods and species for all variables (Table 1), the quality rankings of species were consistent [coefficients of Spearman rank correlations of 0.65, 0.49, 0.59 and 0.59 for CP, NDF, ADF and DMD respectively (significant at $P < 0.001$)] across years. Therefore, significant year×species interactions seem to result from differences in order of magnitude and not from differences in species ranking.

Conclusion

Species choice, lenient cutting intensity and nitrogen fertilizer application are strategies that increase forage quality with a potential positive impact on ruminant performance.

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Forage selection and animal performance of grazing heifers on semi-natural fen grassland

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Abstract

Grazing is a suitable management to maintain the biodiversity and habitat functions of restored fen grasslands. Nutritional requirements of the grazing livestock need to be considered and selective grazing might occur. In order to analyse the selective grazing and actual energy intake by grazing cattle, we implemented a pasture experiment on a percolation mire site in the northeast of Germany. On a 29-ha pasture, 23 heifers had free access to either mineral or fen areas. Standing crop and faeces of cattle were sampled biweekly from May to September at 24 points along a transect. Plant samples were analysed by NIRS. The nitrogen content of the faeces was used to estimate the digestibility of the forage selected by the grazing cattle. Sedge-dominated areas of the fen section were not visited for grazing before the grass-dominated sections of the moraine parts were depleted. This behaviour limited the degree of energy selection and thus the daily liveweight gain of the heifers. These findings show the limits of energy selection on large-scale semi-natural fen pastures and provide a reason to modify the continuous stocking system.

Keywords: animal performance, semi-natural grasslands, forage selection, forage quality

Introduction

Restored fen grasslands in many parts of Europe contribute to biodiversity (Koch and Jurasinski, 2014), supply habitat functions and have potential to store carbon (Dierssen and Nelle, 2006). Grazing has been described as a suitable management tool to maintain these important ecosystem services in general (Middleton *et al*., 2006). However, at the regional level, appropriate grazing practices have to be developed to meet the challenges of maintaining the natural resources while establishing socio-economic sustainable land-use systems in detail (Ostermann *et al*., 1998). In northeast Germany, large-scale and continuous grazing with cattle was regarded as a key tool to fulfil both requirements. Economic success of this low-cost strategy depends to a great extent on the animal performance per head since stocking rate is limited by restrictions. On semi-natural fen grassland the animal performance, a function of amount and quality of forage intake, is to a great degree determined by the botanical composition and the phenological stage of the standing crop (Bockholt and Buske, 1997). Under continuous stocking, the ability of the single animal to graze selectively is the most important adaption mechanism to meet the nutritional demands (Strodthoff, 2002). This is especially important in periods of decreasing nutritive value of the sward.
This study aimed at analysing the degree of selective grazing and actual energy intake and related animal performance of grazing cattle during the grazing period. Results contribute to a better understanding of the processes underlying animal performance on continuously grazed semi-natural fen grasslands.

Materials and method

The grazing experiment was located on a river valley segment consisting of a percolation mire with an adjacent moraine slope, in northeast Germany. From 2011 to 2013, a 34-ha pasture was continuously stocked with 23-27 heifers. Stocking densities were 0.84, 1.03 and 1.08 livestock units (LU) per ha for the grazing seasons 2011, 2012, and 2013, respectively. Heifers had free

access at all times to the moraine slope or fen areas. However, in midsummer 2011 a flooding event prevented access to the peat parts for the remaining time of the grazing season. All animals were equipped with digital earmarks allowing for their identification at an automatic weighing system. Data were used to calculate the daily liveweight gain. The standing pasture crop was sampled biweekly from May to September at 24 points along a transect which included peat and mineral sections. Oven-dried forage samples (65 °C) were ground to 1mm and analysed by NIRS (MPA Bruker®) for nutritive value parameters including ELOS (enzyme soluble organic matter). In accordance with the reference standard methods, crude ash and a sample subset selected using spectral information were additionally analysed according to fit the NIRS calibrations to the experimental data set. Digestibility of the offered pasture growth was calculated in compliance with Weißbach *et al.* (1999). Additionally, we collected fresh cattle faeces biweekly and analysed the nitrogen content. This allowed us to estimate the digestibility of the selected forage according to Schmidt *et al.* (1999). The selectivity was graphically presented as the distance between the digestibilities of offered and realized forage using statistical and graphic tools of SigmaPlot 11.0 ®.

Results and discussion

Trends of forage digestibility over the three observed grazing seasons are presented in Figure 1. Except for the first grazing season 2011, the well-known general trend of decreasing forage values with grazing duration from spring to autumn became obvious.
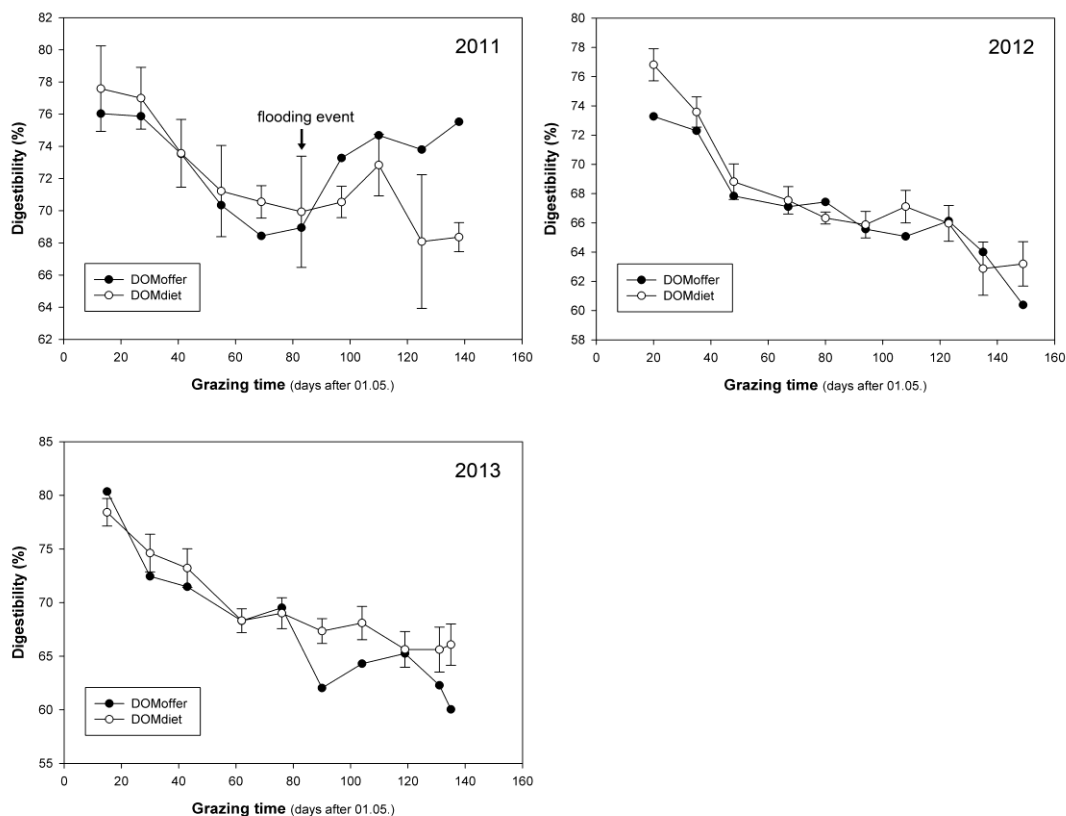
Figure 1. Digestibility of the organic matter of the standing crop (DOM_{offer}) versus digestibility of the pasture diet (DOM_{diet}) at each of the three grazing seasons. Mean values are derived from 24 forage samples (DOM_{offer}) and from 6 faecal charges (DOM_{diet}). Error bars indicate standard deviations of the DOM_{diet} - means.

A flooding event in midsummer of 2011 prevented the cattle from grazing in these sections. This explains the paradox of negative selection for energy in the first experimental year. Periods with higher DOM_{diet}-lines are characterized by successful selection of the offered

pasture growth for digestibility. Such periods can be observed mainly at the beginning of the late summer and to the end of the grazing season (Figure 1).

Compared to the results of Strodthoff (2002), degree of forage selection for high energy content in our experiment was generally smaller than expected. Probably this was due to the dimension of our large-scale pasture and the strong vegetation gradient within. The initially avoided plants at Strodthoff's small-scale pasture were mainly grasses. At our experimental site the fen sections were dominated by sedges, which provide much lesser potential for energy selection. Sedge-dominated areas were not visited for grazing until the grass-dominated sections had been depleted. Furthermore, long pathways and the anxiety to leave the herd seem to counteract an efficient diet selection. We did not observe a clear relationship between the degree of energy selection and the daily liveweight gain over the complete grazing season (data not shown). However, there are strong indications that the degree of energy selection becomes especially important in late summer and autumn.

Conclusion

This investigation shows the magnitudes and limits of energy selection on large-scale semi-natural fen pastures and it provides a reason for modifying the widely used system of continuous stocking. Forcing the cattle into the sedge-dominated fen areas in late spring by fencing would provide both higher digestibility of the standing crop and a better selection base in the late summer under a free-access situation.

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Breed type differences in hoof volume in beef suckler cows

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Abstract

Trampling by cattle can be an effective way of creating establishment sites for plant species of conservation interest, but can also lead to unwanted sward damage. Hoof size and shape could potentially differ between traditional and modern breeds of cattle, in turn influencing their relative impact upon soil and/or vegetation. In this study linear measurements were used to estimate hoof volume for two contrasting breeds of suckler cow: Limousin-cross cows (modern, high performance breed type) and Belted Galloway cows (native breed type). Data were collected for 20 Limousin-cross cows and 10 Belted Galloway cows. The hoof volumes of the fore and hind feet of the Belted Galloway cows were less than the equivalent values for the Limousin-cross cows. However, the smaller body size of the Belted Galloways meant the calculated load on the hooves was similar for the two breeds (0.371 vs 0.399 kg live weight cm^{-3} for the Limousin-cross and Belted Galloway cows respectively; s.e.d. 0.0180 kg live weight cm^{-3}; ns). The results suggest that smaller breeds with smaller feet may have less impact on vegetation and soil types sensitive to damage and erosion, but that larger breeds with larger hoof volumes may be more appropriate where disturbance is desirable.

Keywords: cattle, disturbance, conservation grazing

Introduction

Grassland communities are increasingly recognized as being disturbance-dependent. Trampling, particularly by cattle, can be an effective way of creating establishment sites for plant species of conservation interest within semi-natural vegetation communities (Mitchell *et al.*, 2008). However, poaching (i.e. damage caused to the sward exposing excessive bare ground) contravenes the 'Standards of Good Agricultural and Environmental Condition' and can lead to substantial environmental losses, particularly on carbon-rich soils. Managing the impact of cattle hooves on grassland is therefore both an agricultural and environmental issue. Hoof size and shape could potentially differ between different breed types of cattle, in turn influencing their relative impact upon soil and/or vegetation, and thus their suitability as conservation grazers. In this study correlations between linear measurements of the hoof and hoof volume (Scott *et al.*, 1999), together with body mass, were used to explore the potential impact of different cattle breeds.

Materials and methods

The surface area of the sole of cattle feet rarely represents the surface on which weight is borne (Scott *et al.*, 1999), and hence measurements were made which would allow hoof volume to be calculated. These measurements were made within a week of the hooves being inspected and trimmed as required, to avoid results being distorted by over-grown claws. Data were collected from 20 Limousin-cross cows (modern suckler type) and 10 Belted Galloway cows (native suckler type). For each animal, measurements were made on the digits of the left fore-foot and the right hind-foot (two digits per foot). The data collected per digit were: a) the distance along the proximal border of the coronary band from abaxial groove to flexure of the dorsal surface (CorBand), b) the distance from the abaxial groove, along the distal border or weight-bearing region of the claw, to the point of the toe (Base), and c) the height of the abaxial

groove from the proximal border of the coronary band to the base of the claw (AbaxGr). Hoof volume was then calculated using the formula below (Scott *et al.*, 1999):

Volume (cm^{-3}) = (17.192 × Base) + (7.467 × AbaxGr) + (45.270 × CorBand) – 798.5

The live weight of the cattle was also recorded.

Results and discussion

The hoof volume of both the fore and hind feet of the Belted Galloway cows were less than the equivalent feet of the Limousin-cross cows (Table 1). For both breed types, front hooves were larger than hind hooves, with 61% and 58% of the animals' total volume being found in the front hooves of the Belted Galloway and Limousin-cross cows respectively. These results correspond well to the finding that the forelimbs of cattle bear approximately 60% of the body weight (Fessl, 1968).

Table 1. Hoof dimensions and volume of contrasting breed types of suckler cow.

	Limousin-cross	Belted Galloway	s.e.d.	Prob
Front foot				
CorBand (cm)	14.0	14.2	0.42	ns
Base (cm)	28.8	25.8	0.96	**
AbaxGr (cm)	26.0	23.0	0.73	***
Volume (cm^{-3})	521	456	32.7	*
Hind foot				
CorBand (cm)	12.2	11.9	0.39	ns
Base (cm)	25.8	23.4	0.93	*
AbaxGr (cm)	23.5	19.9	0.78	***
Volume (cm^{-3})	371	287	27.2	**

The Limousin-cross cows were significantly heavier than the Belted Galloway cows (650 vs 589 kg; s.e.d. 22.0 kg; $P<0.05$). However, the calculated load on the hooves of each breed was similar for the two breeds, at 0.371 and 0.399 kg live weight cm^{-3} for the Limousin-cross and Belted Galloway cattle respectively (s.e.d. 0.0180 kg live weight cm^{-3}; ns).

Conclusions

The smaller hoof volume for the same load suggests that smaller breed types such as the Belted Galloway may have less impact on vegetation and soil types sensitive to damage and erosion. In contrast, larger breed types with larger hoof volumes may be more appropriate where disturbance is desirable.

Acknowledgements

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Changes in Koniks' diet due to vegetative season, years and social behaviour

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Abstract

Extensive grazing of horses is conducted in many regions of the Northern hemisphere in order to maintain plant communities with high nature values as well as enabling the recovery of breeds of wild horses. The aim of these studies was to examine the influence of changes in the vegetative season, and of years and herd behaviour, on the percentage share of chosen groups of plants grazed by horses. The studies were conducted in the Biebrza National Park situated in north-eastern Poland. Direct visual observations of grazing of Polish primitive horses (Koniks) from two herds were made during three months (early April, late June and August) in 2009 and 2010. During observation, the first ten bites of the sward made by a chosen mare every 5 minutes were separated into the bitten species. Koniks ate mainly sedges; only in June grasses dominated in their diet with *Carex* sp. The share of specific groups of plants being grazed by Koniks is significantly influenced by weather conditions in the year, which changed the availability of species, and by the season itself, as well as by social behaviour of the animals.

Keywords: diet of horses, grazing, Koniks, sedges

Introduction

The extensive grazing of horses is conducted in many regions of the Northern hemisphere in order to maintain plant communities with a high nature values as well as enabling the recovery of breeds of wild horses. These animals could have an ability to graze plants with low utility value (Prache *et al*., 1998). It is well known that, during the vegetative season, horses show high selectivity towards grazed habitats (Archer, 1973, Crane *et al*., 1997). Simultaneously, although horses prefer mainly grasses and others monocotyledonous species, their preferences towards plant species changes due to many biotic (e.g. availability, quality and growth stages of particular species), abiotic (e.g. season) and individual (e.g. experience) factors (Bailey and Provenza, 2008). Koniks - Polish primitive horses - are descendents of tarpan. Though Koniks graze on wetlands, there is still not enough knowledge about their grazing preferences in this environment. The aim of these studies was to examine the influence of changes in vegetative season, years and behaviours of herds on the percentage share of chosen groups of plants bitten by horses.

Material and methods

The studies were conducted in the Biebrza National Park situated in northeastern Poland, where Koniks have been kept since 2004. Polish primitive horses stayed there in two herds - named after the names of the stallions: Mrok (M) and Limanek (L). Direct visual observations of grazing horses were made by an observer during three months (early April, late June and August) in 2009 and 2010. Every herd was observed for three days in each term in two three-hours periods: during 07-12 a.m. and 4-8 p.m. During observation, the first ten bites of the sward made by a chosen mare every 5 minutes were assigned to bitten species. A random (different) mare was chosen each day from the herd, with no repetitions. The collected data set was analysed with a mixed model that used REML methods (procedure MIXED in SAS 9.3.). Corrected averages and the least significant differences (LSD) were used for the interpretation of gathered data.

Results and discussion

The significant influences on the percentage share of grasses and sedges among plants bitten by horses were both their availability (years) and behaviour of the herds (Figures 1 and 2). In 2010 - a year in which inundation lasted longer than in 2009 - conditions were favourable for species connected with wet habitats, and therefore the Koniks grazed *Carex* sp. more often than in the first year of observation (Figure 1). Among plants bitten by horses from the M herd, it was found that there was about 15% higher percentage share of grasses, simultaneously with a lower share of sedges (Figure 2). It resulted from the larger territory of this herd and more frequent observation of horses grazing on little mid-forest meadows where *Poacae* sp. are dominant in the sward. The L herd stayed mainly in communities situated near Woźnawiejski Canal, where sedges dominated.
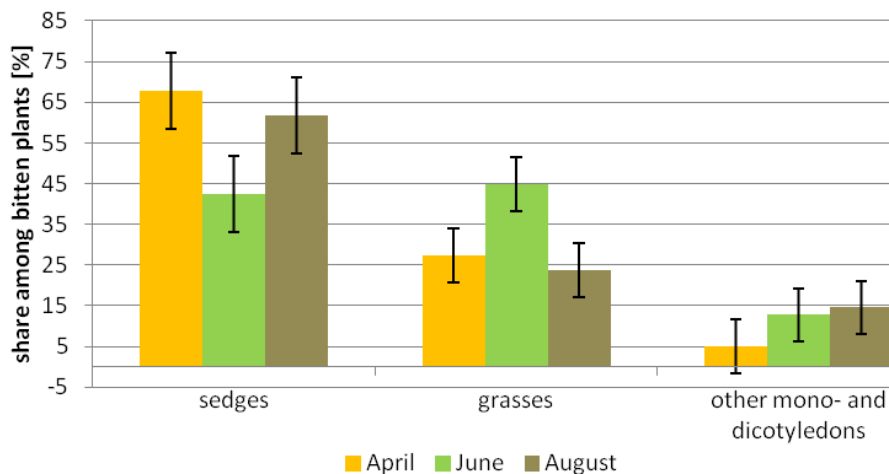
Figure 1. Percentage share of groups of plants in Koniks' bites during vegetative season (two-year average; I = confidence interval, $P \leq 0.05$)
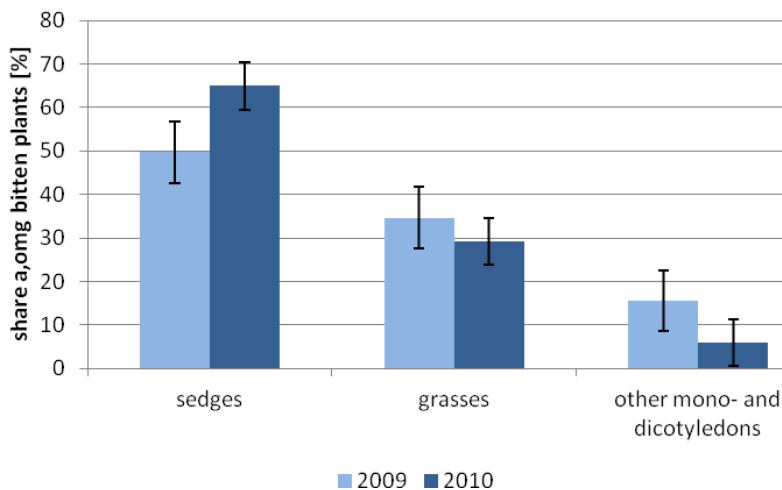
Figure 2. Percentage share of groups of plants in Koniks' bites (in years 2009-2010; I = confidence interval, $P \leq 0.05$).
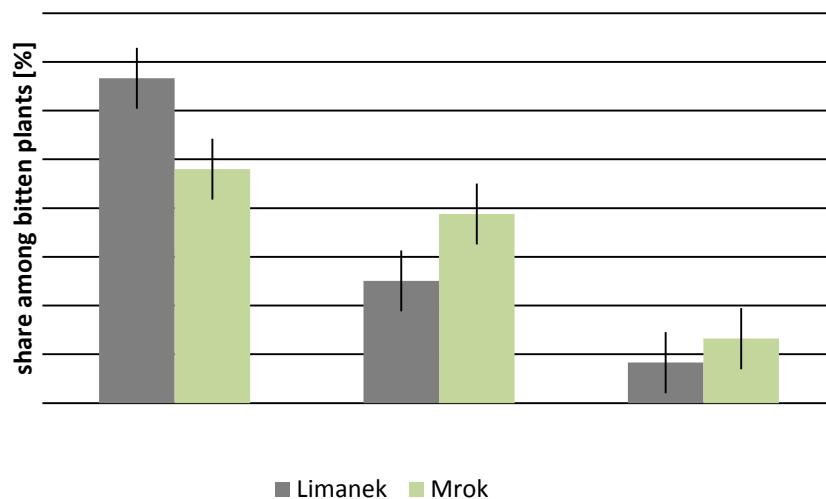
Figure 3. Percentage share of groups of plants in Koniks' bites split by herd (I = confidence interval, $P \leq 0.05$).

Previous research has showed that Koniks could reduce undesirable species successfully, e.g. some sedges (Musielak and Rogalski, 2006; Chodkiewicz and Stypiński, 2011). It should be assumed that, as suggested by Vulink (2001), with better availability grasses would contribute a greater part in the diet of the Koniks.

Conclusion

The share of specific groups of plants being grazed by Koniks is significantly influenced by weather conditions in the year as this changes the availability of species, and by the season itself, as well as by social behavior of the animals. Sedges are dominant in the diet of the Koniks' at the beginning and at the end of the vegetative season, and this may result from delayed growth of grasses.

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Long-term stability of sward patch structure under different intensities of cattle grazing

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Abstract

In low-intensity grazing systems, selective defoliation by the grazing animals can lead to a mosaic pattern of short, frequently grazed, and tall, rarely grazed patches. If the spatial pattern of these patches is stable over time, this may result in divergent development of botanical composition and nutrient cycling. Using sward height data from permanent quadrats, we tested the hypothesis that, under continuous cattle stocking, patch grazing can create sward structures that are stable not only over the short term, but also over several years. For three grazing treatments with different stocking rates, the transitions between three sward height classes (short, medium and tall) were quantified for three time scales: seasonal, interannual and long-term transitions. With the exception of long-term transitions under the highest stocking rate, transitions between sward height classes were non-random, confirming the initial hypothesis of short- and long-term patch stability. A stable sward height pattern may enhance biodiversity and has methodological consequences for studying extensive pastures.

Keywords: patch-grazing, spatial heterogeneity, sward structure, stocking rate

Introduction

In low-intensity grazing systems, selective defoliation by the grazing animals together with positive feedbacks between defoliation frequency and forage quality can lead to a mosaic pattern of short, frequently grazed patches and tall patches where defoliation intensity and frequency are low (so-called 'patch-grazing', Adler *et al.*, 2001). If the spatial pattern of these patches is stable over time, divergent development of botanical composition and nutrient cycling is to be expected.

To assess patch stability in a long-term cattle grazing experiment, we employed a classification with temporally variable class boundaries to account for seasonal and annual differences in sward productivity. We assumed the existence of two functionally different patch types, frequently grazed ('short') and rarely grazed ('tall'). As their height distributions, however, overlap at intermediate sward heights (Rossignol *et al.*, 2011), we included an intermediate sward height class to cover this sward height range.

Using biannual sward height data from seven successive years, we tested the hypothesis that under continuous stocking with cattle at a range of stocking rates, patch grazing can create sward structures that are stable not only over the short term, but also over several years.

Materials and methods

An experiment comparing three grazing treatments in a triplicate randomized block design with a paddock size of 1 ha, was established in 2002 at the experimental farm Relliehausen of the University of Goettingen, located in the Solling Uplands 40 km northwest of Goettingen. The paddocks are grazed by heifers under continuous stocking with three grazing intensity treatments defined by target sward heights: moderate grazing (MG), target sward height 6 cm; lenient grazing (LG): target sward height 12 cm, and very lenient grazing (VLG), target sward height 18 cm (prior to 2005: 12 cm). Target sward heights are maintained in a put-and-take system with bi-weekly sward height measurements. Average stocking rates were 670, 370 and

250 kg ha^{-1}year^{-1} in the treatments MG, LG and VLG, respectively, in the period 2005-2011. Since 2002, no fertilizers have been applied and swards were not cut. For further details, see Isselstein *et al.* (2007).

From 2007 to 2013, compressed sward heights (CSH) were measured each spring before grazing, and each autumn at the end of the grazing season, at 10 randomly located permanent quadrats of 1 m² size per paddock. Per quadrat, five measurements were made, using a rising plate meter with a circular aluminium plate weighing 200 g and with a diameter of 30 cm. Mean values per quadrat were calculated. At every measurement date, each quadrat was classified into one of three sward height classes: short, medium or tall. For each date, the class boundaries were based on the 33rd and 67th percentiles of the CSH at the 90 quadrats, with short: CSH ≤ 33rd percentile, medium: 33rd percentile < CSH < 67th percentile and tall: CSH ≥ 67th percentile. To quantify the long-term stability of patches, the frequencies of transitions between sward height classes were calculated for all quadrats per grazing treatment. Three time scales were considered: seasonal transitions (spring to autumn within each year), interannual transitions (autumn to next autumn) and long-term transitions (autumn 2007 to autumn 2013). The null hypothesis that sward height class at any time is independent of sward height class at a defined previous time was tested separately for each grazing intensity for seasonal, yearly and long-term transitions, using Fisher's exact test as implemented in the Software R (R Development Core Team, 2008).

Results and discussion

Over all sampling dates, the grazing treatment with the highest stocking rate, MG, had the highest proportion of sampling quadrats classified as 'short' (52% compared to 29% in LG and 22% in VLG) and the lowest proportion of quadrats classified as 'tall (15% compared to 40% in LG and 46% in VLG). The proportion of quadrats classified as 'medium' was similar in all grazing treatments (33, 31 and 31% in treatments MG, LG and VLG, respectively). The class boundary between short and medium patches varied between a CSH of 4.2 and 8.5 cm, with a median of 5.8 cm. For the class boundary between medium and tall patches, the median was at a CSH of 9.4 cm, with a range of 7.3-12.3 cm.

Both seasonal and interannual transitions between sward height classes were dependent on initial sward height class (Figure 1). The proportion of short patches becoming tall patches, and, with the exception of grazing treatment MG, the proportion of tall patches becoming short patches, did not exceed 10%. The long-term transitions between sward height classes from autumn 2007 to autumn 2013 only depended on initial sward height in the more extensive grazing treatments LG and VLG. Even at this time scale, transition frequency from short to tall patches and vice versa did not exceed 20% in any of the treatments. Within-year and interannual stability of sward patch structure under continuous cattle grazing have been reported previously (Rossignol *et al.*, 2011; Tonn *et al.*, 2013). Our results indicate that even over a period of seven years, patches can be similarly stable if stocking rates are low, thus confirming the initial hypothesis.

Conclusion

The existence of temporally stable sward height patterns in continually grazed cattle pastures can lead to within-paddock differentiation of vegetation and nutrient cycling between frequently and rarely defoliated patches. Such a functionally heterogeneous mosaic structure has the potential of increasing botanic and faunistic diversity. It should also be explicitly recognized in sampling design of nutrient cycling and biodiversity studies in these systems.
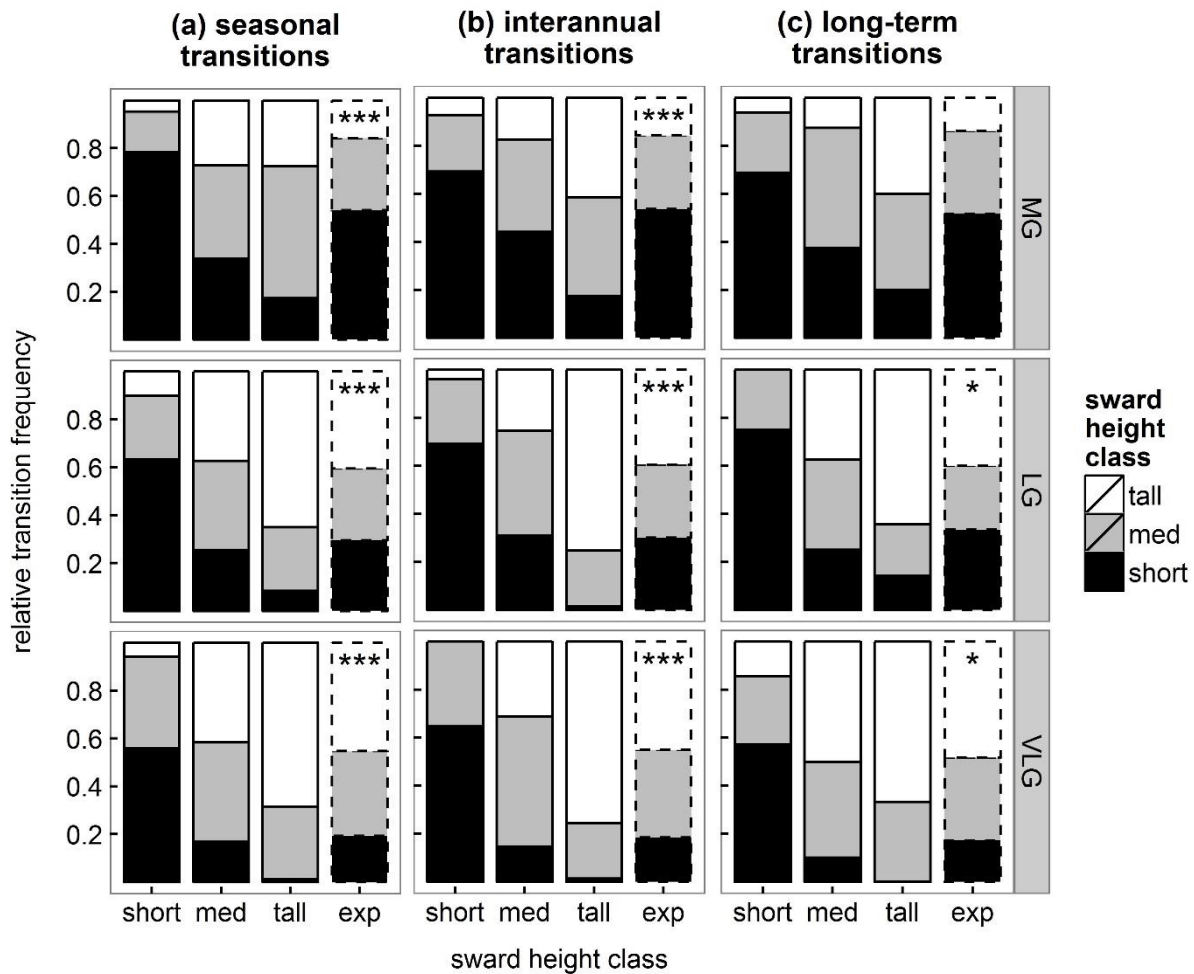
Figure 1: Frequencies with which permanent quadrats belonging to different sward height classes (short, medium or tall) transitioned to each of these sward height classes, compared to the frequencies expected if transitions between sward height classes are random (dashed outline). Asterisks in the upper right corner of each panel mark significant differences between observed and expected frequencies (***: $P < 0.001$, *: $P < 0.05$). Time scale considered: (a) from spring to autumn within each year, (b) from one autumn to following one, (c) from autumn 2007 to autumn 2013. MG, LG, VLG: stocking rates (moderate, lenient and very lenient grazing, respectively).

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Impact of long-term extensive use of permanent grasslands on their provisioning service

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Abstract

In last two decades, changes in grassland management have taken place in Slovakia. The application of agri-environmental measures of the Common Agricultural Policy, which is a main driver of grassland extensification, has resulted in restoration of biodiversity of permanent grasslands and has brought environmental benefits to society as well. Nevertheless, unilateral extensive use of permanent grasslands has begun to have a negative impact on some ecosystem services such as the provision of high-quality forage for cattle. This has consequently resulted in a shortage of beef in human nutrition in terms of Recommended Allowances of Foodstuffs.

Keywords: ecosystem services, permanent grasslands, extensive management

Introduction

Apart from the ecosystem services that permanent grasslands provide for people and the environment, one the main ecosystem services should be the provision of quality herbage for herbivores and a consequent positive effect on human nutrition. The production of surpluses of animal products and environmental damage caused by overgrazing has led to reassessment of the functions and services of permanent grasslands. Although grasslands have always had an important role in the Common Agricultural Policy of the European Union, attention has been drawn to the several ecosystem services, such as grassland habitat maintenance and biodiversity conservation, erosion control and maintenance of soil fertility. Provisioning service of permanent grasslands, in terms of providing high quality herbage, has been suppressed to the background. Nevertheless, extensive use of permanent grasslands supported by agri-environmental payments has resulted in grasslands not being able to provide a full range of ecosystem services, including food security and human nutrition. The objective of this work is to analyse the current state in the use of permanent grasslands and show the possible negative impact of extensive grassland management on their ecosystem services.

Materials and methods

Slovakia is landlocked country in Central Europe with a total area of 49,036 km^2. Slovakia is divided into eight regions, each of which is named after its regional capital. Three regions (Bratislava, Trnava and Nitra) are located in the south-western part of the country, which is under favourable climatic conditions with the highest proportion of productive land dominated by arable cultivation. The other five regions are characterized by the presence of a high percentage of low-quality agricultural land, which translates into a high share of Less-Favourable Areas (LFAs). Currently, the use of permanent grasslands takes place mainly in upland and mountain regions, which with more then 500,000 hectares comprise about 40% of the entire LFAs. The majority of permanent grasslands are found in three regions (Banská Bystrica, Žilina and Prešov). In order to assess current development in provisioning services of permanent grassland, in terms of amount and quality of forage for herbivores, the following indicators were used: grassland area, dry matter yield (DMY), number of livestock units and stocking rate. The basis for the evaluation was data of the Statistical Office of the Slovak

Republic available on-line from RegDat (http://px-web.statistics.sk/PXWebSlovak). The analysis was performed for the period from 2009 to 2011.

Results and discussion

As in other European countries, in Slovakia the number of herbivores, especially cattle, has been decreasing since the 1990s. As in 1990, there were more than 2 million head of cattle in Slovakia; at present the number has decreased to around 463 000 and has been steadily decreasing. A significant reduction of numbers of herbivores has negatively affected the use of grasslands in terms of the area of permanent grassland that is used area, and also the herbage amount and quality. Out of more than 800 000 hectares of permanent grasslands recorded by the Statistical Office of the Slovak Republic, only 507 845 hectares were used in 2011 (Ministry of the Agriculture and Rural Development of the Slovak Republic, 2012).

Table 1. Development of the permanent grassland used area (ha) and dry matter yield (DMY: t ha^{-1})

Region	2009		2010		2011	
	Area	DMY	Area	DMY	Area	DMY
Bratislava	4,807	1.78	4,112	1.65	4,774	2.31
Trnava	8,046	1.71	8,429	1.73	8,742	1.84
Trenčín	40,243	2.19	40,967	2.31	41,813	2.40
Nitra	8,068	1.48	7,653	2.25	8,719	1.97
Žilina	111,815	2.25	110,505	2.38	112,090	2.33
Banská Bystrica	131,719	1.69	123,168	2.11	128,470	1.93
Prešov	137,974	1.66	132,646	1.82	138,618	1.67
Košice	65,665	2.09	61,924	1.91	64,619	2.00
Slovak Republic	508,337	1.86	489,404	2.02	507,845	2.06

Source: Statistical Office of the Slovak Republic

Table 1 shows the stabilization of the permanent grassland used area at national level for the three successive years, 2009-2011. Regarding the use of permanent grasslands in regions, there is evidence of a small increase of permanent grasslands in lowland regions (Trnava and Nitra) whereas the grassland used area has decreased in the Banská Bystrica mountain region by 2.5%. The analysis of stocking rate (Table 2) identified a quite logical location of farming systems in Slovakia, with intensive livestock farming in lowland regions and, conversely, extensive use of permanent grasslands in regions with the highest share of LFAs and important grassland habitats. However, the figures on dry matter yield (Table 1) indicate extensive use of permanent grasslands in both favourable lowland and less-favourable upland and mountain regions. Despite the findings on the positive effect of species-rich permanent grasslands on both quality and nutritive value of animal products, an assessment on the impact of extensive grassland management on herbage quality at selected farms in Slovakia showed that herbage did not meet the nutritional and energy requirements of dairy and beef cattle (Jendrišáková and Kizeková, 2011). The consequence of extensive use of permanent grassland is that concentrates have to be used on the majority of cattle farms in Slovakia, which is problematic if environmental and economic sustainability is also considered. Chrastinová and Burianová (2012) also support these findings and reported loss-making performance of cattle farming since 2008.

The steadily decreasing cattle density in upland and mountain regions has led to a sharp decline of carbon input from grazing animals, which has resulted in a decrease of soil organic carbon stocks on permanent grassland, in comparison with arable land (Bančíková *et al.*, 2013). These findings indicate that long-term extensive management of permanent grasslands could negatively affect some of their supporting services.

Table 2. Development of number of herbivores and stocking rates, years 2009-2011

Region	2009			2010			2011		
	Herbivore LU		Stocking rate of herbivore LU ha^{-1} permanent grasslands	Herbivore LU		Stocking rate of herbivore LU ha^{-1} permanent grasslands	Herbivore LU		Stocking rate of herbivore LU ha^{-1} permanent grasslands
	Cattle	Sheep, goats, horses		Cattle	Sheep, goats, horses		Cattle	Sheep, goats, horses	
Bratislava	19,069	749	4.1	19,016	710	4.8	19,949	667	4.3
Trnava	92,045	1,304	11.6	91,573	1,378	11.0	90,007	1,278	10.4
Trenčín	56,117	7,736	1.6	56,739	7,791	1.6	56,655	7,874	1.5
Nitra	79,715	4,224	10.4	79,541	4,269	11.0	78,527	4,291	9.5
Žilina	92,933	18,428	1.0	92,349	18,947	1.0	92,737	19,057	1.0
Banská Bystrica	100,955	28,886	1.0	101,453	30,355	1.1	99,472	30,333	1.0
Prešov	115,084	18,620	1.0	117,788	18,899	1.0	115,062	18,571	1.0
Košice	61,892	10,611	1.1	62,287	11,506	1.2	61,016	11,322	1.1
Slovak Republic	617,810	90,558	1.4	620,746	93,855	1.5	613,425	93,393	1.4

Source: Statistical Office of the Slovak Republic, own calculation

The most alarming result of the loss-making cattle farming has been a reduction in the consumption of meat and dairy products. In 2009, 2010 and 2011, the annual consumption of beef and veal meet was 4.4, 4.3 and 3.7 kg per inhabitant in Slovakia, which is only 24% of amount in the Recommended Allowances of Foodstuffs (Sitárová, 2010; Ministry of the Agriculture and Rural Development of the Slovak Republic, 2012).

Conclusion

In Slovakia, extensive management of permanent grasslands in both favourable and less favourable areas for nearly the past two decades has lead to degradation of their provisioning and supporting services.

Acknowledgements

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Herbage yield and quality of a limestone grassland managed differently for 30 years

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Abstract

In an observational study over 31 years, the goal was to investigate the effect of mulching vs. mowing and the influence of fertilization (no fertilization vs. different N-P-K and P-K levels) on plant diversity in limestone grassland. Here, the management effects on herbage yield and quality were examined. Yields increased with the level of P-K or N-P-K fertilization, indicating N and P limitation at the site. Low P-K fertilization led to the highest plant diversity of all treatments. It increased the yield by 1.2 t ha^{-1} compared to no fertilization and resulted in comparable metabolizable energy (ME) content. Since it meets both agricultural and nature conservation goals, the requirement for zero-fertilization of nature protection sites should therefore be reconsidered.

Keywords: utilization, fertilization

Introduction

Limestone grasslands are among the most species-rich habitats in Europe and often comprise a multitude of endangered species (Niemelä and Baur, 1998). Therefore they are under nature protection, implying for the farmers that their management has to ensure the maintenance of the species richness (Fauna-Flora guideline 1992). From agricultural aspects, the management of these grasslands of low productivity is not profitable. For this reason, diverse limestone grasslands have declined to a large extent, in particular due to management changes or abandonment (Niemelä and Baur, 1998; Steiner, 2011). Above others the nutrient limitation in limestone grasslands is one reason for their high diversity (Köhler *et al.*, 2001). On the other hand, forbs and legumes rely on a sufficient P and K supply (Magyar *et al.*, 2008). Therefore, there might be fertilizer levels that both facilitate plant species richness and increase herbage productivity and quality. In this paper, the effects of mowing in comparison with the less labour-intensive mulching, and the influence of different fertilizer levels on herbage yield and quality are analysed.

Materials and methods

The site is located in the nature protection site 'Filsenberg' in Mössingen (Swabian Alb, federal state Baden-Württemberg, Germany) at 780 m above sea level. The mean yearly temperature is 6.0 - 6.5 °C, and annual mean precipitation is 850 mm. The soil is a calcareous brown-soil with a depth of around 30 cm, and the texture is sandy or clayey loam over limestone. The vegetation at the start of the observational study was a *Gentiano vernae-Brometum* (KUHN 1957), mown once a year. From the middle of the 20th century onwards it received low dosages of P fertilizer ('Thomasmehl': $Ca_3(PO_4)_2$ (Ca_2SiO_4) containing Fe, Mn, Mg and Cr) or mineral mixed fertilizer.

In 1983, an observational study with varied utilization and fertilization was conducted on plots of 126 m² in size. The treatments were: 1) mulching (Mul); 2) mowing without fertilization (M); 3) P-K 10-16 (M+PK1); 4) P-K 16-64 (M+PK2); 5) N-P-K 10-10-16 (M+NPK1); 6) N-P-K 20-20-32 (M+NPK2); and 7) N-P-K 40-16-64 (M+NPK3; P: P_2O_5, K: K_2O. All values are in kg ha^{-1} a^{-1}). In early spring, plots were yearly fertilized with mineral fertilizer (N fertilizer

'Kalkammonsalpeter': about 80% $H_4N_2O_3$; P fertilizer 'Novaphos': 23% P and 8% S; K fertilizer 'Kornkali': 40% K_2O, 6% MgO, 3% Na, 5% S). In this paper, treatment effects on dry matter yield and metabolizable energy (ME; GfE, 1997) are shown. Since there was spatial and temporal pseudoreplication (i.e. treatments were not replicated and the same plots were surveyed repeatedly), statistical analyses were not possible.

Results and discussion

Differential management did not affect the P and K content of the soil: in all plots the plant-available P content of the soil maintained a very low level (CAL extractable P_2O_5: 2.0 ± 0.5 in 1986, 1.4 ± 0.5 in 2012) throughout the study, while the K content sank from moderate (20.1 ± 3.0) to low values (9.9 ± 0.5; CAL extractable K_2O; values are means ± standard deviations across plots in mg 100 g^{-1} dry soil; sampling depth 0-10 cm) in all plots.

Herbage yields varied within treatments during the study period. This was ascribed to yearly differences in precipitation. Yields of M+PK1 and of the unfertilized plot were up to 3.5 t ha^{-1} a^{-1}, which is in the range found for limestone grasslands in other studies (Ryser *et al.*, 1995; Hochberg and Zopf, 2011; Karrer, 2011). Mulching and M+PK1 reached comparable yields, somewhat larger than those of mowing without fertilization. Hochberg and Zopf (2011) found alternately mown and mulched plots to be more productive than mown plots, an effect likely due to the nutrient return via the mulch layer. The herbage yield was generally increased by fertilization, indicating a limitation of both N and P at the site. The highest productivity was achieved with M+NPK3. In this treatment the proportion of grasses increased during the trial period; on average between 2008 and 2013 a grass proportion of 85% was reached, compared to 55% in the mown unfertilized plot. In grass-based swards, generally higher herbage yields are reached than in forb-based swards (Magyar *et al.*, 2008).
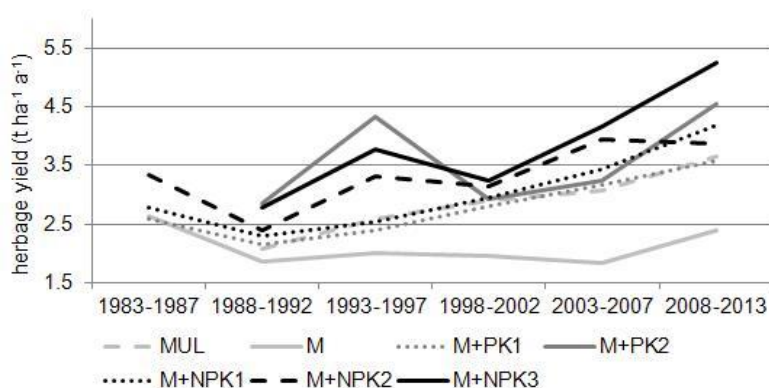
Figure 1. Development of the herbage yield in periods of 5 or 6 years (2008-2013).

The herbage energy content, lying in the range found for other limestone grasslands (Hochberg and Zopf, 2011) at the start of the observation, decreased considerably in all plots after twenty years. This was related to an increase in the grass proportion (data not shown). After 22 years, management measures differed in their effect on vegetation composition. M+PK1 proved to be the treatment that facilitated plant diversity the most (Briemle and Tonn, 2008). The plots with highest dicot proportions, the unfertilized mown plot and M+PK1, resulted in a higher ME and N content of the herbage than the other treatments (Table 1). This is ascribed to the lower decline in herbage quality in dicot- compared to grass-based swards (Briemle *et al.*, 1991). The P and K content in the biomass were positively related to the applied amount of P or K fertilizer. Thus the fertilizer outbalanced the potential positive effect of high legume and forb proportions on the mineral content of the forage.

Table 1. Metabolizable energy (ME) and N, P and K content in the herbage (mean values for the respective time periods; herbage analyses for the determination of ME were not available before 1989). na: no data available, as M+PK2 and M+NPK3 did not start until 1991. For treatment abbreviations see Methods section

	ME (MJ kg⁻¹ DM)		N (g kg⁻¹)		P (g kg⁻¹)		K (g kg⁻¹)	
	1989-96	2005-13	1983-87	2008-13	1983-87	2008-13	1983-87	2008-13
MUL	8.6	8.3	18.6	14.7	1.2	1.4	14.8	17.3
M	8.9	8.7	18.2	15.9	1.4	1.5	13.9	15.0
M+PK1	8.8	8.5	18.5	15.8	1.4	1.8	14.6	16.1
M+PK2	8.4	8.1	na	15.9	na	2.1	na	20.5
M+NPK1	8.7	8.3	18.4	14.5	1.4	1.9	14.7	17.1
M+NPK2	8.8	8.3	18.5	14.2	1.5	2.2	14.8	17.9
M+NPK3	8.7	8.1	Na	13.8	na	2.2	na	20.0

Conclusion

Treatment M+PK1 considerably increased the herbage yield and reached a level of herbage quality comparable to that of the unfertilized mown plot. As M+PK1 meets both agricultural and nature conservation goals, the requirement for zero fertilization, often advocated for nature protection sites, should therefore be reconsidered.

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BIOECOSYS: towards the development of a decision support tool to evaluate grassland ecosystem services

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Abstract

As underlined by the Millennium Ecosystem Assessment report (2005), it is necessary to take into account and preserve all the functions and connected services associated with ecosystems. Therefore, agroecosystems such as grasslands, covering near one-fifth (19.5%) of the European territory – rising to 50% of the Walloon utilized agricultural area – and providing important ecosystem services, have to be studied and managed as multifunctional units, opening new opportunities for valorization. This will allow the answering of societal expectations oriented towards more sustainable agriculture and improved use of natural resources. In this context, the final and main goal of the BIOECOSYS project is the development of a specific methodology and decision support system for the quantification and valuation of ecosystem services provided by the grassland ecosystem linked to its history and management, to its soil and climate context, to its location in the landscape and in the socio-ecosystem. To reach this objective it is necessary to produce integrated knowledge at the different levels of organization of grassland agroecosystems: (1) to quantify ecosystem services to integrate them in decision-making processes, and (2) to give a value (economic or not) to the services provided to guide decision-making choices. A first result of the project, a provisional scheme of grassland ecosystem functioning in relation to the services provided, is presented.

Keywords: agroecosystem, management, valuation, DSS, ecosystem function

Introduction

During the last decades, the over-exploitation and degradation of ecosystems has been recognized in different reports (Millennium Ecosystem Assessment, 2005). In parallel, growing demand for agricultural products, international awareness of biodiversity loss (e.g, the 1992 United Nations Rio Earth Summit) and climate changes have led to reconsideration of agroecosystems. The current challenge is to maintain or restore these ecosystems, enabling them to produce enough food but also services to improve the environment and human well-being. This must be done not only by avoiding pollution but also by maintaining and increasing 'ecosystem services' such as public goods (maintenance of water and air quality) or environmental services (maintenance of biodiversity, carbon sequestration) (Lemaire *et al.*, 2005; FAO, 2007). To reach these goals, agriculture must rely on the increasing scientific knowledge about agroecosystems, especially considering the ecosystem-services concept, which is very useful in the agricultural and public policies establishment (Lamarque *et al.,* 2011). The concept of agroecosystems multifunctionality, translated into ecosystem services, provides a new framework to drive researches necessitating genuinely inter-disciplinary approach (Hervieu, 2002; Lemaire *et al.*, 2005). This concept also represents a key element in the development of ecologically intensive agriculture which aims to optimize the use of agroecosystem functionality to produce more while preserving, or even enhancing, its environmental services (Bonny, 2011). In Europe, grasslands are essential ecosystems representing near one-fifth (19.5 %) of the European territory – rising to 50% of the Walloon

utilized agricultural area – and providing important ecosystem services such as forage production, erosion and resources regulation, *etc*. Nevertheless, grasslands are actually threatened by land conversion to crops and face a significant pressure. Despite the demonstrated abilities of grassland systems to provide numerous ecosystem services (Amiaud and Carrère, 2012), for the diversity of grassland agroecosystems there remains a need to provide precise information on these services linked to their management and location (Puydarrieux and Devaux, 2013).

BIOECOSYS and its objectives

In order to allow the inter-disciplinary approach that will be necessary, the expertise of several units of the Walloon Agricultural Research Centre will be mobilized in order to carry out this project. These units are (1) Farming systems, Territories and Information technology Unit, (2) Plant protection and Ecotoxicology Unit, (3) Soil fertility and Water protection Unit, (4) Food and feed quality Unit, and (5) Crop production systems Unit. The final and main goal of BIOECOSYS is the development of a specific methodology and decision support system for the quantification and valuation of ecosystem services provided by the grassland ecosystem linked to its history and management scheme, its soil and climate context, and its location in the landscape and in the socio-ecosystem. To reach this objective it is necessary to produce integrated knowledge at the different levels of organization of grassland agroecosystems. Several ecosystem services will be quantitatively studied at the field and the landscape scales, where the basic biogeochemical processes are acting, while the valuation of grassland ecosystem services will be evaluated at the regional scale, supporting socio-economic and political decisions (Lemaire *et al.*, 2005; Hein *et al.*, 2006).

Grassland ecosystem services conceptualization: a first output

Early reflections and bibliographic researches have resulted in a first draft of grassland ecosystem functioning in relation to the services provided. Firstly, the CICES classification was examined to identify the different ecosystem services provided by grasslands. We applied the methodology described by Lamanda (2012) to conceptualize the grassland system. These services were connected with the grassland ecosystem functioning. The grassland is schematized on the basis of its three main compartments: (1) the *soil*, (2) the *vegetation cover* and (3) the *faunal composition*, in which various processes are taking place. Several abiotic factors (e.g. topography, landscape, climate, *etc*.) influence the functioning of these processes resulting in chain reactions which alter the supply of ecosystem services. In parallel, agricultural practices have a demonstrated and variable impact on the provision of ecosystem services. These different management methods (mowing rate, grazing intensity, fertilization schemes, *etc*.) and their impacts on grassland ecosystem services have to be modeled in order to allow their integration in the decision support system. This conceptualization frame will be validated by three focus groups mobilizing expertise in different fields interconnected. This will allow us to give a relative importance and an orientation to the different interconnections highlighted.
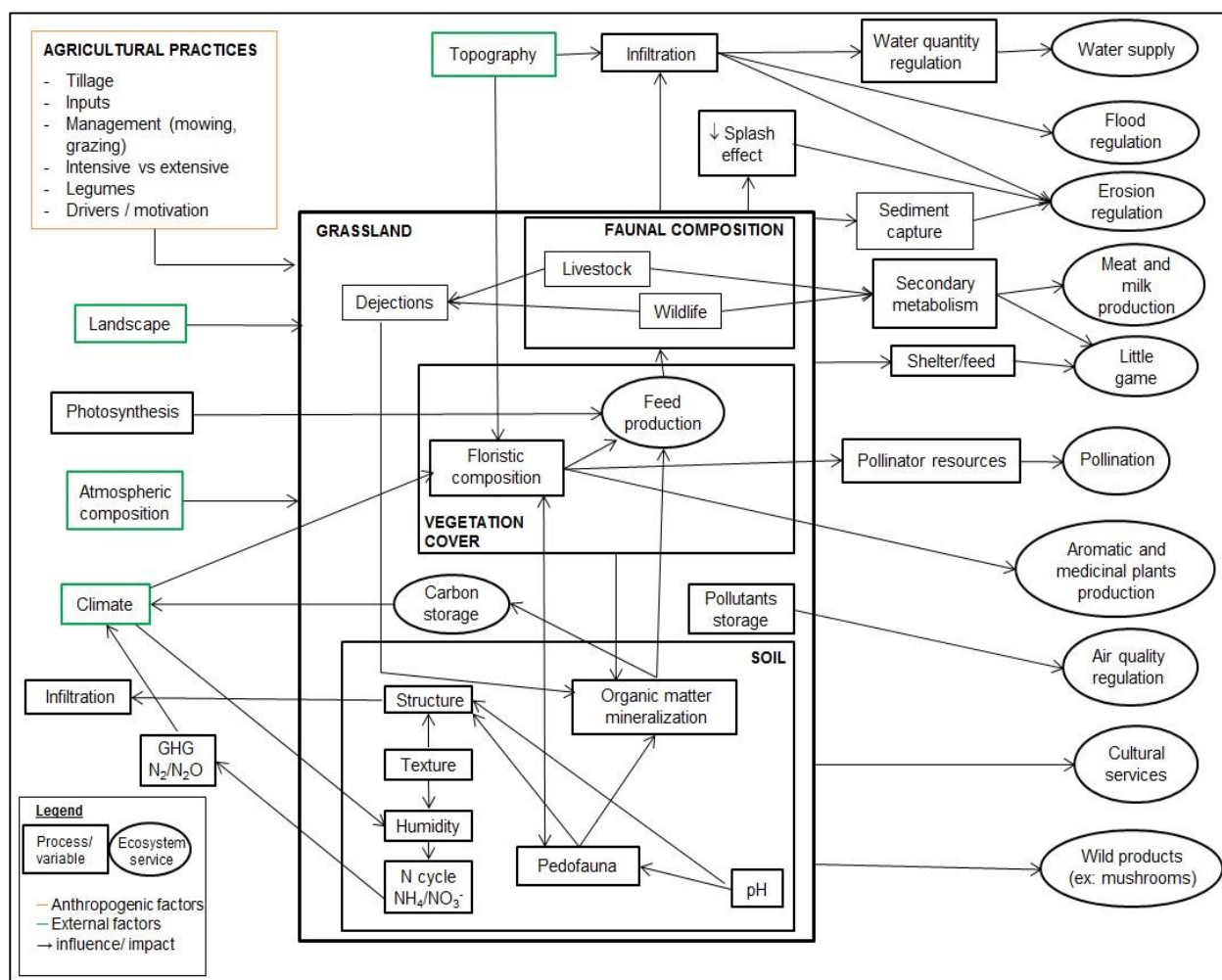
Figure 1: Conceptualization of grassland ecosystem in link to ecosystem services provided

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Grassland biodiversity: how we might meet international commitments

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Abstract

England has very challenging targets to meet the UN Convention on Biological Diversity, whilst aspiring to produce more food. And with a low proportion of publicly owned land this has to be achieved largely from commercial farmland. Most grassland receives less than 50 kg ha^{-1} of N fertilizer and has high potential for increasing biodiversity as well as greater production. This requires: the 2% of grassland which is species-rich to be better managed and expanded by restoration and creation, for which we have good techniques; other priority habitats, for example grazing marshes, better managed to protect priority birds and other species; very small areas (1%) in corners and margins allowed to grow tall for invertebrate nest sites; winter seed for birds (minimum 2%) which could be provided by allowing ryegrass to set seed; some areas (2-5%) grazed leniently and not topped, to provide habitat for invertebrates which are food for birds; much more grassland sown with legumes and robust herbs, a small proportion being allowed to flower to provide for bees and other pollinators; protection of legumes and herbs by avoidance of overall use of broad-spectrum herbicides, controlling thistles and other injurious weeds by targeted treatment.

Keywords: Grassland, biodiversity, habitat, invertebrates, birds, legumes.

Introduction

England has a very high population density exceeding 400 km^{-2} and one of the lowest proportions of publicly owned land in Europe (Cahill, 2002) so a high proportion of the biodiversity and ecosystem services has to be delivered from privately owned agricultural land. This is one of the reasons why English agri-environment schemes are among the most ambitious and best-funded in Europe. The UK government is committed to challenging actions to meet the UN CBD Aichi targets, whilst at the same time aspiring to produce more food (DEFRA, 2011). Here I consider how this might be achieved from grassland.

Fertiliser inputs

Inputs to grassland fell from a peak in the late 1980s to less than half of that by 2012 (Table 1). Only 21% receives >100 kg ha^{-1} and 41% receives zero N fertilizer (Table 2). It might be expected that declining N-use would be compensated by increasing reliance on legumes, but UK sales of clover seed fell from 900 t in 1981 to 400 t in 2004 (FERA, 2005). Since then data have not been available.

Table 1. Use of fertilizer nitrogen, phosphate and potash (kg ha^{-1}) on grassland in England and Wales 1973-2012. (British Survey of Fertilizer Practice, 2012)

Year	Nitrogen	Phosphate	Potash
1973	85	34	22
1986	135	22	33
2002	85	18	24
2012	54	8	11

Table 2. Use of fertilizer nitrogen (kg ha^{-1}) on grassland in England and Wales: percentage of grassland area by field application rate (British Survey of Fertilizer Practice, 2012).

kg N/ha	0	1-49	50-99	100-199	>200
%	41	15	21	18	3

The current agri-environment scheme, Environmental Stewardship (ES), has options which do not allow fertilizer N. Approximately 400,000 ha (9%) of grassland is in such options and a further 450,000 ha (11%) is in options which allow a maximum of 50 kg ha^{-1} although often in practice receives less, or even zero.

So it is clear that most grassland in England is managed quite extensively, even when not in agri-environment agreement.

Biodiversity, and production

Table 3 shows that 2% of grassland is rich in plants, and also in scarce invertebrate species. A further area of semi-natural grassland, mainly of coastal and floodplain grazing marsh, is of particular importance for scarce birds and other target species. Lawton *et al*. (2010) concluded that the most important actions required to protect and increase biodiversity are to better manage and enlarge these habitats, and to create or restore new core areas. We have good understanding of such management, and of soil suitability and techniques for restoration (Peel *et al*., 2012) from existing grassland and arable land. Restoration and creation have been targeted in ES at land with low phosphorus status and/or other limiting characteristics (Peel and Diack, 2007) and there is evidence that grassland meeting the minimum threshold of priority habitat can be created, sometimes in less than 10 years (Natural England, 2013), although it may take *c*.100 years to fully develop. Livestock output from priority habitat is low but individual livestock performance and health on species-rich neutral and calcareous grassland can be good, even from commercial breeds (DEFRA, 2014a).

Table 3. Summary of biodiversity of enclosed grassland in England

Descriptor	Area ('000 ha)	% of grassland	Typical higher plant species (m-2)	Probable fertiliser N (kg ha-1)	Value for biodiversity	
					Current	Potential
Priority habitat	104	2	16-40	0	Very high	Some – condition of most can be improved.
Semi-improved grassland	1453	33	9-15	0-50	Moderate to very high	High – introduce more species, diversify sward structure.
Improved grassland	2856	65	<10	0-300+	Low-moderate	High – introduce legumes and robust herbs, allow to flower occasionally, no overall herbicide sprays.
	4413	100				

Sources of area data: UK National Ecosystem Assessment (2011); Countryside Survey (2009).

But increasing priority habitat towards 3% of grassland is not enough to reverse the declines of formerly common pollinators, other invertebrates and farmland birds, nor in the longer term to enable them to adapt to climate change. This requires core areas to be better connected (Lawton *et al*., 2010) and grassland has a key role to play in making farmland more permeable to wildlife. Further plant species, and more diverse structure, can be added to the 33% of grassland that already has moderate botanical diversity. And on the 65% that is species-poor a wider range of grasses, more legumes (e.g. *Trifolium pratense, T. repens, Lotus corniculatus)* and robust herbs *(e.g. Cichorium intybus, Plantago lanceolata, Centaurea nigra, Sanguisorba minor)* can be sown (DEFRA, 2014b). Current output from improved and semi-improved

grassland is quite variable: herbage production and nutritive value could be improved by greater reliance on legumes (Lüscher *et al.*, 2013).

Conclusions and opportunities

With a well-resourced agri-environment scheme, priority habitat could be better managed and expanded to protect plant species and some specialized invertebrates. But many invertebrates and farmland birds require a range of resources on which to feed and breed at a larger scale than can be provided in England by priority habitat alone. Semi-improved and improved grassland, in association with woodland, hedgerows, wetland and arable land, could provide these resources. It would need:

Very small areas (1%), in field corners and margins, allowed to grow tall for invertebrate nest sites. Could be sited to also buffer watercourses.

Winter seed for birds (min 2%). Could be provided from plots on arable areas. Otherwise by allowing ryegrass to set seed (DEFRA, 2010)

Some areas (2-5%) grazed leniently and not topped, to provide habitat for invertebrates which are food for birds (DEFRA, 2013).

Much more grassland sown with legumes and robust herbs. A proportion must flower to provide for bees and other pollinators, which provide pollination services to crops.

Protect legumes and herbs: control thistles and other injurious weeds by targeted treatment, not overall use of broad-spectrum herbicides.

Because grassland is currently relatively extensively managed, yet use of legumes is limited, these changes would not necessarily reduce, and might increase, livestock production.

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Resilience of Mediterranean ecosystems: tree and management effects on variability of herbaceous pastures in a dry year

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Abstract

The presence of trees and adaptative management techniques play an important role within Mediterranean agroforestry ecosystems. Dehesas are good examples of Mediterranean 'High Natural Value' agriculture and produce important natural resources. Our work focuses on the influence of trees and main management techniques (stocking rate and site quality) on yield and alpha diversity of a Dehesa in Central Spain. We analysed total yield, Richness and Shannon-Wiener indexes within the herbaceous pastures under 16 holm oaks (*Quercus ilex* ssp *ballota* (Desf.) Samp) located in different management zones, in a very extreme dry year, with phosphoric fertilization, intensive stocking rate, medium stocking rate, and high soil quality or low soil quality. Tree canopy had a significant influence on herbaceous yield and alpha indexes (both were higher outside canopy influence). Phosphoric fertilization also had a significant effect on herbaceous yield and alpha diversity. Tree presence, livestock and soil management were shown to be important factors modifying the characteristics of herbaceous pastures in Mediterranean agroforestry systems, even in extreme climatic conditions.

Keywords: Mediterranean management, yield, alpha diversity, tree-grass interaction

Introduction

The presence of trees plays an important role providing a wide range of important ecological functions and ecosystems services within Mediterranean agroforestry ecosystems (Manning *et al*., 2006). Within the influence area of their canopy and roots, trees can change pasture species composition, structure, spatial distribution and biomass (Scholes and Archer, 1997). These agroforestry systems are associated with different common techniques of management (e.g. intensive grazing, fertilization) affecting the extensive yield-diversity of pastures and tree-grass interactions. Grazing by large herbivores alters the composition and biomass of grasslands (Bartolome and McClaran, 1992). Fertilization with minerals suitable for organic agriculture can be a management and conservation tool for semi-natural grasslands (Pãcurar *et al*., 2012). Mediterranean Dehesas are good examples of Mediterranean 'High Natural Value' agriculture in Europe, assigned as 'Site of Community Importance' (43/92/EEC Directive), and they are producers of important natural resources. Therefore, considering management decisions over tree-grass interaction in ecosystem dynamics and functional studies is a key research line. Our work focuses on the influence of trees and main management techniques (stocking rate and site quality) on yield and alpha diversity of a Dehesa in Central Spain.

Materials and methods

The study area was located within a Dehesa in Central Spain (39°N, 5°W; 350 m asl). The climate is continental Mediterranean and soils are sandy (>80% sand), acidic, and poor in organic matter (<1%). Sixteen holm oaks were selected for the study, distributed in four zones (5 ha each) with different management procedures (phosphoric fertilization; intensive stocking rate; medium stocking rate and high soil quality, hereafter MH; and medium stocking rate and low soil quality, hereafter ML). All of them were grazed by sheep. The average canopy radius

(standard deviation) of trees is 6.2 m (1.4). At each tree, we located six sampling frames (50 × 50 cm), considering three positions according to the proximity of the trunk (beneath crown: 0.5 radius; edge crown: 1 radius; and beyond canopy's influence: > 1 radius). In total, 96 sampling units were studied. In each one, we analysed floristic composition and abundance by species or morphospecies, and calculated Richness and Shannon-Wiener indexes to measure alpha diversity. In addition, we mowed aboveground herbaceous biomass at ground level at the end of May; this was taken immediately to the laboratory where it was dried at 80ºC until stabilized at constant dry weight. We obtained the total dry matter at each sampling unit. We used generalized linear mixed models (GLMM) to study relationships between total yield or alpha diversity measures and independent factors (type of management and distance from the trunk). The model averaging approach (Burnham and Anderson, 2002) was used. We used R programming environment (Version 3.0.2, R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org) for data processing, analysis and presentation of results.

Results and discussion

Mediterranean areas are associated with a high topo-edaphic and climatic variability, and specifically inter-annual rainfall variability (Gea-Izquierdo *et al.*, 2010). It means that some years could be very extremely dry, as our studied 2012 year (298.1 mm; 596.7 mm average of last 20 years). However, in spite of these dry conditions, we found differences in biomass and composition of the herb layer due to the presence of trees and different types of management. A total of 70 different plant species or morphospecies was recorded at the estate. Beneath crown positions there were significantly lower values for total yield (166.1 kg ha^{-1}± 107.6) than at the edge-crown positions (233.3 kg ha^{-1}± 155.0), which showed lower values than beyond-crown positions (494.8 kg ha^{-1}± 217.6) (Table 1). Within Mediterranean silvopastoral systems and depending on climatic conditions, the influence of trees can be opposite (McClaran and Bartolome, 1989). Both alpha indexes were significantly higher outside canopies than under the influence of tree canopies (beneath- and edge-crown projection) (Table 1). However, trees provide different species composition, increasing the whole-system diversity in Mediterranean agroforestry systems (Marañón, 1986). The fertilized plot showed higher values for total yield and Richness than zones with intensive stocking rate and MH zones (without differences between them). Shannon-Wiener index was higher at the fertilized plot than MH and MP zones (Table 1). Phosphoric fertilization had a significant effect on yield and alpha diversity of the herbaceous layer, but year effect (rainfall) mainly determined the effect of phosphoric fertilization. We did not find differences for Shannon-Wiener index between fertilized and intensive grazing zones. The tree random effect scarcely modifies the yield or alpha diversity average of the herb layer among trees.

Conclusion

High annual rainfall fluctuations within Mediterranean agroforestry systems, as dehesas, provide large differences on interactions between ecosystems components. Even at extremely dry conditions we found notable differences due to the presence of trees and different management techniques. The tree effect (under, beneath and edge canopies influence) provided different species composition increasing herbaceous diversity of whole system. Some management practices as P_2O_5 fertilization or intensive sheep grazing had a significant effect on yield and alpha diversity of herbaceous layer. The presence of trees and appropriate management techniques maintain and conserve the quality of dehesas, increasing the resilience of the Mediterranean ecosystem.

Table 1. Summary of the top GLMM fitted (delta<2) to analyse the differences on total yield and alpha diversity indexes. Coeff.: estimated coefficient average; SE: standard error average.

Number of top models	Response variable	Fixed effects	*Importance*	Factors	*Coeff.*	*SE*	*P*
1	[a]Total dry matter	Management	1.00	MH	-40178	14963	0.007
				ML	31347	27094	0.247
				I	-32255	15588	0.039
		Distance	1.00	Ecotone	14394	6797	0.034
				Outside	198869	45078	<0.001
1	[b]Richness	Management	1.00	MH	-0.406	0.117	0.001
				ML	-0.160	0.109	0.143
				I	-0.226	0.111	0.042
		Distance	1.00	Ecotone	0.170	0.108	0.118
				Outside	0.520	0.101	<0.001
2	[c]Shannon	Management	0.53	MH	0.137	0.044	0.002
				ML	0.088	0.040	0.027
				I	0.070	0.039	0.070
		Distance	1.00	Ecotone	-0.075	0.042	0.075
				Outside	-0.188	0.040	<0.001

[a]Error distribution (link): Gamma (power, l=2); Deviance explained: 15.0%; Dispersion: 0.31; AIC= 1230.6
[b]Error distribution (link): Poisson (log); Deviance explained:19.0%; Dispersion: 0.25; AIC= 422.9
[c]Error distribution (link): Gaussian (inverse); Deviance explained:28.5.0%; Dispersion: 0.91; AICmin= 158.9
I: intensive stocking rate; MH: medium stocking rate and high soil quality; ML: medium stocking rate and low soil quality; Ecotone: samplings located at 1 radius of crown projection; Outside: samplings located beyond canopies' influence.

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Soil organic carbon and nitrogen stocks affected by grazing intensity in temperate permanent grassland

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Abstract

Stocks of organic C (C_{org}) and total N (N_t) in soils of grazed grassland ecosystems may be affected by the management intensity. However, respective data for grasslands of the temperate zone are scarce. Soil samples were taken in 0-10 cm, 10-25 cm and 25-40 cm depth from pasture plots of one ha in central Germany which were intensively (mean compressed pasture height (CPH): 6 cm), extensively (mean CPH: 12 cm), and minimally (mean CPH 18 cm) grazed. Because of the large grazing heterogeneity the 1-ha plots of different grazing intensity showed patches of high (18 cm), medium (12 cm), and low (6 cm) CPH. We took soil samples from three soil depths, and three plots as well as three sub-plots of different CPH. In the 10-25 cm soil depth, our results showed significantly higher C_{org} and N_t stocks for extensive grazing compared to minimal grazing, suggesting positive effects of moderate grazing intensity on C_{org} and N_t stocks of the subsurface mineral soil.

Keywords: Grazing, management intensity, C_{org} stocks, N_t stocks

Introduction

The intensity of grassland management varies but it is often driven by the demand for forage production. The type and intensity of management may exert a marked influence on the soil organic C (C_{org}) and total N (N_t) stocks of pastures (Conant *et al.,* 2001). Moreover, the C_{org} stored in grassland soils may be in conjunction with the N_t storage, which is important for the productivity of grassland ecosystems (Conant *et al.,* 2001). However, previous studies show varying effects of grazing on C_{org} and N_t; many of these differences may be the result of variations in climate and soil properties (Derner *et al.,* 2006). Consequently, it is not possible to extrapolate from global data sets or from data of arid or semi-arid environments to pasture conditions in the temperate zone. For the latter, there have been only few studies which have investigated the effect of the grazing intensity on soil C_{org} and N_t stocks. Consequently, the objectives of our study were to analyse in a first step the effects of three different grazing intensities on the stocks of C_{org} and N_t in three different soil depths of a permanent grassland in central Germany.

Materials and methods

The study site is located in central German approximately 60 km NE of Goettingen (Lower Saxony) at 250 m above sea level. The mean annual temperature is 8.2 °C and the long-term average annual precipitation is 879 mm (Şahin Demirbağ *et al.,* 2008). The experimental field site is a mesotrophic, moderately species-rich hill grassland on a brown earth pelosol with an average number of plant species of 10.9 m^{-2} (Şahin Demirbağ *et al.,* 2008). The vegetation type is a Lolio-Cynosuretum (Scimone *et al.,* 2007).

A grazing experiment with cattle and no input of fertilizers, pesticides or cutting was established in spring 2005 with following treatments:
intensive grazing pressure (IGP), target mean compressed pasture height (CPH) of 6 cm
extensive grazing pressure (EGP), target mean CPH of 12 cm

minimal grazing pressure (MGP), target mean CPH of 18 cm

The different grazing intensities were determined by measuring the CPH in the vegetation period at intervals of two weeks and, if necessary, the stocking rates were subsequently increased or decreased to ensure the targeted CPH. Each treatment was carried out in three replications by dividing three blocks into three paddocks of 1 ha, with different grazing intensities (i.e., intensive, extensive, minimal). Because of the selective cattle grazing, the paddocks did not show a homogeneous CPH. To adress this issue, the paddocks were further subdivided into patches of different CPH (i.e., low: 0-6 cm, medium 6-12 cm, high 12-18 cm). Soil samples were taken from the patches (low, medium, high: two pseudo-field replicates) within each paddock (intensive, extensive, minimal) in three depths (0-10 cm, 10-25 cm and 25-40 cm). The samples were sieved <2 mm and stored at 4 °C. The concentrations of total C (C_t) and N_t in the bulk soil were determined by dry combustion using a CN elemental analyser (Elemtal Vario El, Heraeus, Hanau, Germany). The inorganic C concentration was measured with the Scheibler method following DIN 19682-13 (2009). The C_{org} content was calculated by substracting the inorganic C from the C_t content. The C_{org} and N_t stocks of the different soil layers were calculated for an equivalent mass of soil as suggested by Ellert and Bettany (1995) to take differences in bulk density of the respective soil layers into account (Jacobs *et al.,* 2010). Statistical analyses were conducted with the statistic software R (R Development Core Team, 2010). The two pseudo-replicates per paddock were averaged to carry out statistical analyses for nine different variants (3 grazing intensities × 3 CPH). For the analyses of the effect of grazing intensity, all six values for one paddock were averaged. The data were analysed with one-way analyses of variance (ANOVA). Effects were considered significant for *P ≤ 0.05*.

Results and discussion

In the surface soil (0-10 cm) C_{org} stocks showed large variability and no significant differences between the treatments with different grazing intensities were found (mean ± standard deviation for the treatments in t ha^{-1} were 34 ± 7 for IGP, 39±10 for EGP and 33±10 for MGP). The C_{org} and N_t stocks in the soil depth 10-25 cm, however, were significantly lower for MGP (C_{org}: 32 ± 6 t ha^{-1}; N_t: 3.3 ± 0.8 t ha^{-1}) than for EGP (C_{org}: 45 ± 11 t ha^{-1}; N_t: 4.2 ± 0.5 t ha^{-1}). These grazing intensities did not show significant differences in C_{org} and N_t compared to IGP (C_{org}: 42 ± 11 t ha^{-1}; N_t: 4 ± 0.8 t ha^{-1}). For EGP, the C_{org} and N_t input due to the stimulating effects of grazing on photosynthetic rates (e.g. stimulation of root growth as well as tiller and leaf, inhibition of stem and flower, incorporation of dead plant material) may have been more pronounced than the destructive effects that grazing exerts on the sward, which is linked with losses of C_{org} and N_t (Frame and Laidlaw, 2011). For MGP, the growth stimulation and also incorporation of dead plant material due to trampling was presumably markedly less compared to EGP.

Conclusion

Extensive grazing pressure with a CPH of about 12 cm seemed to be the best management intensity for the studied pastures in a temperate climate, which established highest C_{org} and N_t stocks in soil.

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Effects of biomass of perennial grasses and legumes on soil carbon

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Abstract

Experiments were carried out with the aim assessing the effects of incorporation of perennial grasses and legumes: red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), alfalfa (*Medicago sativa* L.), timothy (*Phleum pratense* L.) as green manure on the amount of soil organic carbon. Ploughed-in biomass of red clover and alfalfa, second year of usage, provided 2.5 – 2.8 times more organic carbon than ploughed-in timothy, and 2.2 – 3.1 times more than from white clover. The amount of organic carbon depended on the plant's aboveground biomass and root mass (respectively r = 0.999** and r = 0.992**). The C:N ratio of the aboveground biomass of legumes was the lowest (10.0–14.0) and was more favourable for a rapid decomposition than of the aboveground and root mass of timothy (20.0–47.0). After two years of soil usage for crop cultivation, the amount of organic carbon in soil increased by 40.4-52.5% when timothy grass or legumes of the second year of usage were ploughed, and by 20.0–22.2% when grasses of the first year of usage were ploughed. At the end of the study the C:N ratio was favourable for humification of organic carbon.
Keywords: perennial grasses, biomass, organic carbon, C:N ratio

Introduction

Residues of plant aboveground biomass and root biomass are the main source of soil organic carbon (Rasse *et al*., 2005). The rate and duration of accumulation of organic material partly depends on the initial amount of N and C in soil (Velthof and Oenema, 2001). In grassland ecosystems up to 98% of total C is sequestered below ground (in the rhizosphere) and sequestered carbon is less volatile than carbon from above ground (Schlesinger, 1977). Carbon and nitrogen mainly accumulate in the top 10 cm layer, where approximately 80% of plant roots are developed (Davies *et al*., 2001). The aim of this study was evaluate the biological value of biomass of perennial species on soil carbon changes in soils of western Lithuania.

Materials and methods

Field experiments were conducted in western Lithuania at the Vezaiciai branch of the Lithuanian Research Centre for Agriculture and Forestry (55°43$^/$ N, 21°27$^{/'}$ E) in 2002-2007. Two analogous experiments were set up in 2002 and 2003. The soil of the experimental site was *Albi – Edohypogleyic Luvisol,* light loam on medium heavy loam. The agrochemical characteristics of the plough layer were as follows: pH_{KCl} – 6.0-6.1, mobile P_2O_5– 104-199 mg kg^{-1} soil, K_2O – 120-166 mg kg^{-1} soil, N_{total} 0.08-0.11%, C_{org} 0.90-1.05%. The experiments were conducted in the following crop rotation sequence: perennials – winter triticale (*Triticosecale* Wittm.) – spring barley (*Hordeum vulgare* L.). Perennials included: red clover, white clover, alfalfa, timothy. Perennial species were ploughed under as green manure at different stages of development, of and for first year (I) and second year (II) of usage.

The biomass was chopped and shallowly incorporated during the beginning of flowering of legumes and at the beginning of ear emergence of timothy. After two weeks it was then deeply ploughed to 25 cm. No mineral fertilizers or plant protection products were used, in order to determine the biological value of the different preceding crops.

Plant root mass was determined from 10 cm depth by the Katchinski monolith washing method. The mass of all plant residues and aboveground biomass were recalculated into dry matter.

Soil samples were collected before the trial establishment and after ploughing the perennials to below the 0–20 cm depth. Available P_2O_5 and K_2O were determined by the A-L method, total nitrogen by Kjeldahl, organic carbon by mineralizer 'Heraeus'. Statistical analysis was carried out using ANOVA (** in tables indicates significance at $P < 0.01$).

Results and discussion

The amount of aboveground and root mass of the perennial species in the crop rotation depended largely on the biological properties of the plant species and the stage of development (Table 1). Aboveground mass of perennials from the first year of usage was higher than from the second year of the same species by, respectively: 10% for red clover, 33% for white clover, and 45% for timothy. The smallest amount of aboveground mass originated from the timothy of second year.

Table 1. Dry matter yield of biomass, organic carbon and C:N ratio of perennial species in first year (I) and second year (II) of usage

| Perennials | Dry matter yield | | Amount of organic carbon and C:N | | | |
| | Aboveground mass | Root mass | Aboveground mass | | Root mass | |
	$g\ m^{-2}$	$g\ m^{-2}$	$g\ m^{-2}$	C:N	$g\ m^{-2}$	C:N
Timothy I	403.7	721.3	152.2	26	223.8	47
Timothy II	223.7	1016.7	86.4	23	312.7	36
Red clover I	1904.5**	660.0	688.5**	12	233.6	20
Red clover II	1714.2**	1139.3**	637.1**	14	416.3*	22
White clover I	898.89**	374.2	323.0*	14	130.2	23
White clover II	603.5	311.3	231.0	14	108.1	24
Alfalfa II	1051.0**	1799.4**	372.2	10	627.0**	27
$LSD_{0.05}$	317.861	355.558	163.232		147.575	

The greatest amount of root biomass was from perennials of the second year of usage (with the exception of white clover) and the greatest amounts were from alfalfa and red clover, second year of usage. Root biomass from alfalfa and red clover accounted, respectively, for 63.1 and 39.9% of the biomass used as green manure. The smallest amount of biomass (915 g m⁻² of dry matter) was produced by white clover of the second year of usage. Their roots constituted 34% of the biomass used as green manure. The general amount of accumulated organic carbon in biomass of the tested perennials was found to be 35.2-38.6% in aboveground part and 29.5-37.0% in the roots.

The greatest amount of organic carbon was incorporated into the soil by ploughing of red clover (1053 g m⁻²) and alfalfa (999 g m⁻²) of the second year of usage. It was 2.5 – 2.8 times more than by ploughing of timothy for different development stages, and 2.2 – 3.1 times more than by ploughing white clover (Table 1). The amount of organic carbon depended on the plant's aboveground biomass and root mass (respectively $r = 0.999**$ and $r = 0.992**$). Carbon and nitrogen ratio (C:N) varied between plant species and between biomass origin (aboveground part or roots). Clover root biomass C:N ratio was similar, but significantly smaller than that of timothy.

Before the experiments, soil analysis showed organic carbon amounts were from 0.90 to 1.05% (Table 2). At the end of the study, after cultivation of triticale and spring barley, the amount of organic carbon increased by up to 40.4 – 52.5%, when perennials of second year of usage were ploughed under, and by 20.0 – 22.2% after ploughing the perennials from first year of use. Maximum C_{org} increase was delivered from alfalfa and red clover.

Table 2. Changes in organic carbon in soil (0-20 cm) for first year (I) and second year (II) of perennial species (T – timothy, RC – red clover, WC – white clover, A – alfalfa).

Parameter of soil		Perennial species						
		T I	T II	RC I	RC II	WC I	WC II	A II
C_{org} before the experiment		0.95±0.07	1.04±0.08	0.95±0.04	1.04±0.02	0.90±0.03	1.05±0.02	1.01±0.03
	C:N	9.5	10.4	9.5	10.4	9.0	11.7	10.1
C_{org} at the end of experiment		1.14±0.15	1.46±0.20	1.16±0.22	1.53±0.05	1.10±0.18	1.53±0.10	1.54±0.17
	C:N	9.5	11.2	10.5	10.1	9.2	12.7	11.0

Prior to the experiments, the C:N ratio in the soil arable layer was 9.0 – 11.7. After two years the C:N ratio increased where white clover, alfalfa and timothy of second year of usage were ploughed under (respectively by 8.5, 8.9 and 7.7%) and red clover of the first year of usage (10.5%). This resulted because of an increase of organic carbon after cereal cultivation.

Conclusions

The tilled-in biomass of perennial grasses considerably increase the amount of organic carbon in soil and led to its accumulation in the topsoil layer. Maximum amount of sequestered organic carbon was observed after tilling-in of red clover (1053 g m^{-2}) and alfalfa (999 g m^{-2}) of second year of usage, which was 2.5-2.8 times greater than from timothy, and 2.2 to 3.1 times more than from white clover. After two years of crop cultivation the soil organic carbon content increased by 40.4-52.5% when second year perennials were ploughed under, and by 20.0-22.2% when the first year perennials were ploughed under. At the end of the research, the C:N ratio in the arable soil layer was favourable for humification of organic carbon in all variances.

Acknowledgments

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Soil organic carbon characteristics under different intensities of grassland management

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Abstract

The effect of intensive and extensive grassland management on hot-water soluble carbon (C_{hws}) and water soluble carbon (C_{ws}) was monitored. The experimental plots were located at 400 m above sea level on *Cambisol* soil type in the Czech Republic. Three treatments, each applying mineral and organic fertilizers with graded stocking rates and different mowing frequency, were conducted on the permanent grassland. Average C_{hws} values ranged from 573 to 657 mg kg^{-1} and C_{ws} values ranged from 210 to 258 mg kg^{-1}. Hot-water soluble and water soluble carbon were influenced by different grassland management. The greatest amounts of C_{hws} and C_{ws} were measured under the 'medium intensive' management. Intensive management led to a decline in the amount of C_{hws} and C_{ws}.

Keywords: hot-water soluble C, water soluble C, grassland, *Cambisol*

Introduction

Soil organic carbon is one of the most important parts of the soil due to its ability to affect plant growth, both a source of energy and a trigger for nutrient availability through mineralization (Edwards *et al*., 1999). The organic carbon comprises an easily degradable part which can be expressed as hot-water soluble carbon (C_{hws}), and water soluble carbon (C_{ws}). These properties are known as indicators of the amount of available soil C substrate. These properties are studied for their usefulness as soil quality indicators responding to changes in the rhizosphere caused by management practices (Ghani *et al*., 2003). Both parameters can be also influenced by soil type (Uchida *et al*., 2012) and by altitude (Kolář *et al*., 2003). Previous studies have reported about C_{hws} and C_{ws} changes and dynamics influenced by grazing (Haynes, 2000), fertilization (Ghani *et al*., 2003), mowing and mulching (Váchalová *et al*., 2013) in grasslands. The aim of this study was to evaluate the quantitave changes of soil organic carbon under different levels of grassland management (extensive, medium-intensive, intensive).

Materials and methods

The experimental plots were located in the northwest part of Moravia in the Czech Republic. The area is situated 400 m above sea level and the soil is sandy-loam, type *Cambisol*. Annual average air temperature is 7.7 °C and annual rainfall average is 693 mm. The locality is characterized by semi-natural permanent grassland. Three treatments, each applying 3 rates of mineral and organic fertilizers, were conducted on the permanent grassland as shown in Table 1. Experimental plots were arranged in a completely randomized block design with four replications. The plot size was 12.5 m^2. In 2013 soil samples (from a depth 0.05–0.30 m) were taken to determine the content of hot-water soluble carbon C_{hws} (Körschens *et al*., 1990) and water soluble carbon C_{ws} (Váchalová *et al*., 2013).

Statistical data analysis was undertaken using the statistical program SPSS 13.0 for Windows. Analysis of variance (ANOVA) was performed by statistical program. Mean statistical differences (95% significance level) were calculated by Tukey's HSD test ($P < 0.05$). We tested homogeneity of variances by Cochran–C's test. Correlation analysis was also used.

Table 1. Experimental treatments

Treatment	Fertilization	Livestock unit (LU)	Amount of applied N	No. of cuts
Control	without	0	0	2 cuts per year
Extensive	mineral and organic	0.9 LU ha^{-1}	60 kg N ha^{-1}	2 cuts per year
Medium-intensive	mineral and organic	1.4 LU ha^{-1}	90 kg N ha^{-1}	3 cuts per year
Intensive	mineral and organic	2.0 LU ha^{-1}	120 kg N ha^{-1}	4 cuts per year

Results and discussion

The effect of intensive and extensive grassland management on C_{hws} and C_{ws} was monitored. The measured values of C_{hws} and C_{ws} were optimum for this soil type. Kolář *et al.* (2003) reported that optimum value of C_{hws} ranged from 300 to 600 mg kg^{-1}. The values of C_{ws} were positively correlated with the values of C_{hws}. Chosen forms of carbon content showed differences between the treatments (Table 2).

Table 2. Average values of C_{hws} and C_{ws} for the particular treatments. a, b, c - values with the same letter are not statistically different within the column $P < 0.05$

Treatment	C_{hws} (mg kg^{-1})	C_{ws} (mg kg^{-1})
Control	573[a]	210[a]
Extensive	642[b]	238[b]
Medium-intensive	657[c]	258[c]
Intensive	637[b]	234[b]
P	0.035	0.027

The differences in C_{hws} and C_{ws} were significant between control and all fertilized treatments. There were statistically significant differences in C_{hws} and C_{ws} between 'medium intensive' (3 cuts per year, 90 kg N ha^{-1}) treatment and all the other treatments. The content of C_{hws} was higher at the treatment with 'extensive' (2 cuts per year, 60 kg N ha^{-1}) management in comparison to 'intensive' (4 cuts per year, 120 kg N ha^{-1}) management but this trend was not statistically significant ($P = 0.786$). Greater intensity of grassland use (multiple mowing) led to a moderate decrease in C_{hws} and C_{ws}. Duffková *et al.* (2005) also reported about C_{hws} and C_{ws} decrease in connection with a higher frequency of mowing. These results corresponded with findings of Ghani *et al.* (2003) who published that intensive grassland management had negative impacts on C_{hws} content. The greatest content of selected forms of soil carbon was measured at the 'medium intensive' treatment. Intensive management showed decrease of soil carbon.

Conclusion

Our study confirmed a positive effect of 'medium intensive' management on selected forms of soil organic carbon. Hot-water soluble and water soluble carbon were influenced by the different types of grassland management. The greatest amount of hot-water soluble and water soluble carbon was recorded under the 'medium intensive' level of management. Intensive management led to a decline in the amount of hot-water soluble and water soluble carbon.

Acknowledgements

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Efficacy of the agrosteppe method for restoring eroded lands

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Abstract

The steppes have been experiencing severe anthropogenic stress for thousands of years, and they are largely used for arable farming. The growing concern for biodiversity led to the necessity of developing a habitat restoration method which would result in establishment of a plant community totally equivalent to the initial one. The natural process takes up to 150 years, including the undesirable primary stages of forbs' association featuring an excessive propagation of the hazardous allergenic species *Ambrosia artemisiifolia* and other ill weeds. Sod planting has been found ecologically inadequate, because of the uneven sward and strong competition between the sod blocks and the seedlings of the new generation. However sowing a complete species mixture after ploughing was proved efficient. The problem of seed collection conditioned by the differences in ripening time of the target species was solved by harvesting the equal adjacent areas of seed donors in two or three stages at 25-30 days intervals. This technology and the swards created were termed 'agrosteppe'. The typical plant community obtained as a result was stable and self-reproducing already in three years after establishment.

Introduction

Rehabilitating completely destroyed multicomponent natural ecosystems, such as steppes, meadows, prairies and savannas, takes 80-150 years of natural self-remediation via succession (demutation). The recovered steppes (grasslands, *etc.*) should be similar to the natural ecosystems in their composition and flora abundance, as well as in their vertical structure, resistance to anthropogenic stress, economic and aesthetic features such as biological productivity, functionality, and wildlife well-being. On agriculturally used sites this results in similarity to the properties of natural hay and pasture and to the dietary characteristics of the resulting animal products.

Materials and methods

The experimental site of 1100 m^2 (11 ha) is located on eroded lands in the Stavropol Territory of Russia. It is situated on the 2-6° southern slope of undulating terrain. The sandy soil contains only 0.9-1.0% humus; annual precipitation is uneven, up to 450-500 mm. The land was intensively used as a pasture. After the degradation of the natural steppe vegetation, the site was converted into arable area for growing crops such as wheat, maize, sunflower, and grasses for hay. Previously to agrosteppe establishment, the territory was abandoned and inhabited by weed communities with forbs prevailing: *Daucus carota*, *Ambrosia artemisiifolia* and *Artemisia vulgaris*. The height of the upper layer reached 1.5 m and surface coverage amounted to 60%. The direct census was carried out in accordance with Oscar Drude's plant abundance scale on fixed 100 m^2 sites. The four *Poaceae* species made 18% of the plant community; the only two *Fabaceae* representatives amounted to 9%, and the 16 forbs species dominated at 73%. The latter group was mostly represented by harmful weeds, being a hazard both for the crops and for human well-being (*Ambrosia artemisiifolia*). The agrosteppe was established in July 1980. The method has this name because the steppe is reconstructed using conventional

agricultural machinery, tools and techniques for tillage, sowing and harvesting the complex natural seed mixture.

Results and discussion

During the first year after the sowing of the agrosteppe, the typical wildland species were accompanied by approximately 60% of weeds. In the next year, 1982, the diverse steppe perennials successfully competed with them and finally dominated in the swards. Since the third year on the agriculturally created young steppe the sward has been sustainably reproducing itself without human interference. Single forbs are found only on the disturbed sites near animal holes and burrows. The stabilized botanical portrait of the agrosteppe (Table 1) is mainly represented by the grass association: *Festuca valesiaca + Stipa pulcherrima + Filipendula vulgaris*. The upper layer is 0.75 m high, the dominant layer is 0.50 m, and the ground layer is at 0.30 m. The surface coverage is 90-100%. The plant diversity of the agrosteppe has reached 67 species, compared with the 22 of the degraded land. The species abundance is subject to slight natural fluctuations in accordance with the changeable weather conditions resulting in so called 'clover years' or '*Stipa* years', and so on. Like a natural steppe community the established one shows considerable tolerance to the pyrogenic effects and revegetates the next year. In addition, since the very first years of existence, agrosteppe is inhabited by animal species, including pollinators (*Apis* spp., *Bombax* spp., *etc.*), birds, reptiles, rabbits, and later by foxes, and others. Thus, both the phytocoenosis and the zoocenosis of the steppe ecosystem are reproduced simultaneously.

Table 1. Botanical composition of the fully developed agrosteppe (3rd year of life and on)

Poaceae + Cyperaceae (19.4%)			Forbs (73%)		
1.	*Bromopsis riparia*	Sp3	26.	*Achillea nobilis*	Sp2
2.	*Carex michelii*	Sp2	27.	*Achillea setacia*	Cop1
3.	*Cleistogenes bulgarica*	Sp1	28.	*Arenaria serpyllifolia*	Sp2
4.	*Dactylis glomerata*	Sp1	29.	*Dianthus ruprechtii*	Sp3
5.	*Erytrigia intermedia*	Sp1	30.	*Echium russicum*	Sp3
6.	*Festuca pratensis*	Sp2	31.	*Filipendula vulgaris*	Cop2
7.	*Festuca rupicola*	Sp3	32.	*Geranium sanquineum*	Cop1
8.	*Festuca valesiaca*	Cop3	33.	*Hieracium echioides*	Sp3
9.	*Koeleria cristata*	Cop1	34.	*Plantago lanceolata*	Sp3
10.	*Phleum phleoides*	Sp3	35.	*Plantago media*	Sp3
11.	*Poa bulbosa*	Sp3	36.	*Veronica austriaca*	Sp3
12.	*Stipa pennata*	Sp1	37.	*Ranunculus polyanthemos*	Sp2
13.	*Stipa pulcherrima*	Cop2	38.	*Stachys atherocalyx*	Sp3
Fabaceae (9%)			39.	*Salvia tesquicola*	Sp1
14.	*Amoria ambigua*	Sp3	40.	*Thymus marshallianuc*	Sp3
15.	*Amoria montana*	Cop1	41.	*Potentilla argentea*	Sp3
16.	*Amoria repens*	Sp1	42.	*Potentilla adenophylla*	Sp3
17.	*Astragalus austriacus*	Sp2	43.	*Jurinea arachnoidea*	Sp3
18.	*Lotus caucasicus*	Sp2	44.	*Potentilla recta*	Sol
19.	*Medicago minima*	Sp2	45.	*Fragaria viridis*	Sp3
20.	*Medicago romanica*	Sp3	46.	*Euphorbia sequieriana*	Sp2
21.	*Onobryhis arenaria*	Sp2	47.	*Euphorbia iberica*	Sp1
22.	*Trifolium alpestre*	Sp3	48.	*Euphorbia stepposa*	Sp2
23.	*Trifolium pratense*	Sp2	49.	*Cerastium caespitosum*	Sp3
24.	*Vicia angustifolia*	Cop1	50.	*Verbascum phoeniceum*	Sp2

25.	*Vicia tenuifolia*	Sp3	51.	*Centaurea orientalis*	Sp3
			52.	*Tragopogon dasyrhynchus*	Sp3
			53.	*Orchis tridentata*	Sp2
			54.	*Veronica verna*	Sp2
			55.	*Muscari muscarimi*	Sp3
			56.	*Salvia verticillata*	Sp2
			57.	*Verbascum lychnitis*	Sp1
			58.	*Anemone sylvestris*	Sp1
			59.	*Dracocephalum austriacum*	Sp3
			60.	*Paeonia tenuifolia*	Sp2
			61.	*Myositis suaveolens*	Sol
			62.	*Scabeosa ochroleuca*	Sp1
			63.	*Eryngium campestre*	Sp1
			64.	*Poterium polygamum*	Sp3
			65.	*Leontodon hispidus*	Sol
			66.	*Gallium ruthenicum*	Sp3
			67.	*Pastinaca pimpinellifolia*	Sol

Conclusion

The established agrosteppe is perennial like that of the natural zonal steppe, and it is likewise self-sustaining without any human intervention. It becomes a seed donor for ecological restoration of unproductive desertified lands: 1 ha of the donor steppe provide for 7-10 ha of resown territory. This allows recovering low-productive and abandoned land, and reproducing the floral diversity of steppe exponentially.

In two years the weed community is replaced by a typical diverse steppe vegetation with approximately 67 species per 100 m^2, including valuable forage grasses (*Festuca, Koeleria, Bromopsis, Dactylis, Phleum, etc*.), legumes (*Trifolium, Amoria, Medicago, Onobrychis, Lotus, etc*.), and forbs (*Plantago, Poterium, Filipendula, etc*.). The created sward contains a number of medicinal plants (*Thymus, Fragaria, Achillea, etc*.), and conditions the propagation of rare and protected genera such as *Stipa, Anemone, Paeonia,* and *Orchis*.

Zonal strategy for sward renovation by total reseeding based on research results

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Abstract

In recent years, the Research-Development Institute for Grassland, Brasov has carried out experiments in the Fagaras depression, a large area in the centre of Romania. The purpose of the research purposes has been to find the most suitable solutions for improving degraded grasslands in accordance with the area conditions and a lower environmental impact. Based on research results, a zonal strategy for sward renovation using a total reseeding technique was established and extended on to larger areas.

Keywords: grassland, research solutions, reseeding, Fagaras depression

Introduction

Grasslands are an essential element of sustainable farming systems, in terms of animal welfare, fodder provision, soil quality and the best use of less-productive land. The experimental fields were located in representative areas of the Fagaras depression, allowing a high degree of extrapolation of research results. Investigation has focused on establishing a favourable interaction between crop and livestock systems in this area, with good results in terms of natural-resource use (Mocanu *et al.*, 2011). This region covers an agricultural area of over 111,000 ha, of which 49,925 ha (45%) are grasslands, at altitudes of 450–650 m above sea level (a.s.l.). There are especially mixed farms (crops and livestock), the most economic farming system way in the current conditions. Except for eutric and carbonatic soils located in the Olt Floodplain, the other soils in the Fagaras Depression (about 110,000 ha, or 90% of the basin area) have, in addition to nutrient deficiencies, some limitations induced by high acidity and large amounts of exchangeable Al_3^+ *etc*. The forages of permanent grassland are poor in terms of yield and quality because of different stages of degradation, with multiple reasons for this. Therefore, to achieve high yields with high nutritional value of feed and high conversion efficiency in livestock products, it is necessary to apply measures to improve these pastures.

Materials and methods

The area under intervention is located in the Dragus village, Fagaras Depression, which is property of the local association of animal breeders. It is on a permanent pasture, 25 ha in area, at 505 - 530 m a.s.l. The area is in an advanced stage of degradation caused by a bad management (absence of annual clearing and maintenance work, invasion of worthless species, irrational grazing, and no fertilization or correction of soil acidity *etc*.). The soil agrochemical study highlights an area with high acidity and with a poor nutrients supply. Based on this soil agrochemical study, research results and the current area conditions, a zonal strategy for sward renovation by total reseeding has been established.
The works applied to improve these degraded grasslands were as follows:
- liming 4 – 6 t ha^{-1} agricultural limestone, in autumn 2012;
- total destruction of the old sward by heavy disc harrow (two perpendicular passes) in the autumn of 2012;
- seedbed preparation by disc harrow (two passes perpendicularly) in the spring of 2013;

- seeding with valuable perennial grass and legume mixtures, simultaneously with rolling before and after sowing, using a special grass sowing machine made by the Research-Development Institute for Grassland, Brasov (Mocanu and Hermenean, 2013);
- fertilization with complex fertilizers N15P15K15, 350 kg ha^{-1}.

For tilling, the disc-harrow alternative was chosen due to the thickness of fertile topsoil. Because the improved grassland is to be used in a mixed system, grazing and cutting, a complex seed mixture was selected. This seed mixture is suitable for local area conditions and consists of (see Mocanu *et al.*, 2013): *Festuca pratensis* 8 kg; *Festuca arundinacea* 13 kg; *Dactylis glomerata* 5 kg; *Trifolium pratense* 2 kg; *Trifolium repens* 2.5 kg; *Lotus corniculatus* 3.5 kg; in total 34 kg seed ha^{-1}.

Results and discussion

Because of the terrain topography, the reseeded area is divided into three plots**,** as follows: 18 ha in plot I, 5.5 ha in plot II and 1.5 ha in plot III.

There was good establishment of the forage crop, with good ground cover of sown species, average of cover was from 88% on plot I, to 92% on plot II (Table 1). The average feed yield after the first harvest by mowing the three plots was 3.9-4.3 t DM ha^{-1}, while the output of the control plot was 1.0-1.3 t DM ha^{-1}. The second cycle consisted of using cattle grazing, average yield ranging between 1.4 – 1.7 t DM ha^{-1}. Therefore, in the first year of the sward establishment, total feed yield of improved pasture was between 5.3 and 6.0 t DM ha^{-1}.

Table 1. Percentage cover and botanical composition of sown and control plots.

Species	Sown plot, participation rate, %			Control plot, %
	I	II	III	
Percentage cover of sward	88	90	92	100
Grasses, of which:	74	53	35	92
Dactylis glomerata	43	30	25	-
Festuca arundinaceea	12	12	5	-
Festuca pratensis	7	5	3	-
Festuca rubra	6	3	1	10
Agrostis tenuis	6	3	1	60
Poa pratensis	-	-	-	10
Nardus stricta	-	-	-	12
Forage legumes, of which	23	45	64	-
Trifolium pratense	11	30	45	-
Trifolium repens	5	6	10	-
Lotus corniculatus	7	9	9	-
Other grasses	3	2	1	8
TOTAL	100	100	100	100

Some soil samples for each variant (plot I and II with three replicates, and plot III with two replicates) were analysed before (control plot) and after intervention. As a result of liming, strong acid reaction of the soil has improved noticeably, especially in the 0-10 cm depth, soil pH status increasing on average of 1.27 units, from 4.74 (high acidic) to 6.01 units (low acidic) in all three plots.

After fertilizer application, there was an increase of more than 3 times in the phosphorus content (6-19 mg kg^{-1} P_{AL}) in the 0-10 cm depth. In addition to some assessments of botanical composition (Table 1) there were determinations of the main nutritional parameters (Table 2): crude protein, crude fibre, cell wall constituents (NDF, ADF, ADL), crude ash and dry matter digestibility (DMD) and organic matter (OMD), by infrared spectroscopy technique (NIRS).

Table 2. The main nutritional parameters of feed obtained on plots, % DM.

No.	Variant	Crude Protein %	Crude Ash %	Crude fibre %	NDF %	ADF %	ADL %	DMD %	OMD %
1	Control plot	8.60	7.30	40.80	73.90	45.00	4.20	45.20	44.20
2	Plot I, average	15.54	9.46	29.13	57.93	33.10	3.33	69.43	66.26
3	Plot II, average	16.36	9.70	29.26	56.56	33.13	3.70	66.76	63.06
4	Plot III, average	18.25	10.65	25.80	52.40	30.90	3.55	71.45	68.90
5	Total, average	16.72	9.94	28.06	55.63	32.38	3.53	69.21	66.07

Crude protein content (CB %) was in the range 15.54-18.25%, which is characteristic of quality forage, while the control plot had small protein content (8.6%). The constituent content of cell walls: NDF, ADF, ADL had values characterizing a forage with medium to high feeding value. The digestibility coefficients of the analysed forage samples have presented also values for a good quality of forages provided by reseeded pastures.

Conclusion

The establishment of productive grassland depends greatly on providing the right conditions for seed germination, and for seedling and root growth, with the final aim to develop a dense sward. For a successful action, it is necessary to remove the reasons that have caused the grassland became of low quality. To know these reasons, several analyses concerning the stationary area conditions, soil characteristics, botanical composition of the old vegetation, and the climatic conditions are carried out. Further, based on analysis and research results, the best solution for improving the degraded grassland needs to be decided, taking into account the following factors: appropriate tillage system for destruction of the old vegetation, reseeding period, seedbed preparation, basic fertilization, seed mixture choice, seeding machinery and post-sowing management.

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Magnesium content in soil and selected layers of upland grassland biomass

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Abstract

Investigations were aimed at determining the magnesium (Mg) content in soils and selected layers of plants, depending on the management method (hay production, grazing) and nitrogen fertilization level with basic PK fertilization. A four-year period of management and fertilization resulted in lowered content of available Mg in soil samples collected from all objects. Increasing nitrogen fertilization led to elevated Mg concentration in the soil of the experiment used for hay production, whereas an opposite relationship was revealed in the experiment with grazing. Mineral fertilization caused the highest increase in Mg concentration in the harvested plants. The lowest extractable Mg was in the root layer between 0 and 5 cm for the $N_{120}P_{18}K_{66}$ treatment in both experiments.

Keywords: hay, grazing, fertilization, magnesium content

Introduction

Magnesium deficiency poses a serious problem in soils and plants of permanent grasslands (Szewczyk *et al*., 2007). The highest deficit of this macroelement occurs in acid soils developed from loose and weakly clay-sands, and in shallow, skeletal soils (Sapek, 2008), usually covered with grass vegetation used for pastures. The Mg content in soil and sward herbage is affected by nitrogen fertilization (Kulczycki, 2006).

The research aimed at determining the Mg concentration in soils and plants of grasslands depending on the methods of their management and nitrogen fertilization level, against the background of phosphorus and potassium treatment.

Materials and methods

The research comprised two experiments conducted in 2007–2010. The experiments were located on a permanent grassland (49º 47' 23'' N; 19º 50' 47'' E; elevation 470 m a.s.l.), on acid brown soils developed from sandstone with the composition of light loam.
The first experiment was used for hay production and pasture, in which the first regrowth was cut at the full-earing stage of the dominating grass species, and the other two regrowths were grazed by a flock of mountain sheep. The other experiment was utilized as pasture, grazed by mountain sheep four times every year. The grazing was in quarters and lasted for three days for the first and second regrowths, and two days for the third and fourth regrowths.
A randomized block design with four replications was used. Two fertilizer variants were considered in the experiments according to the scheme in Table 1. Control treatments were cut and grazed but did not receive mineral fertilizers. Dry matter yield of the sward used for hay production was assessed by cutting plants from the 12-m^2 plots, whereas the sward used as pasture was cut from an area of 1 m^2 of each plot prior to each grazing. The plant samples from the replications were mixed and dry-mineralized in a muffle furnace at 450 ^{O}C. Magnesium was extracted by hot dilution in nitric acid (1:1). The contents of Mg in plant material were assessed by the ICP-AS method on JY 238 Ultrace. Prior to the onset of the experiment and after its completion, turf samples were collected from an area of 30×30 cm area and to a depth of 15 cm from all treatments of both experiments in order to determine the aboveground plant (stubble) mass, root mass and its distribution in the 0–5 cm and 5–15 cm layers.

Table 1. Mineral fertilization scheme

Management	kg N ha^{-1} (doses before follow regrowths)	kg P ha^{-1} (single dose in spring	kg K ha^{-1} (doses before follow regrowths)
Mixed	80 (48 + 32) 120 (60 + 30 + 30)	18	66 (33 + 33)
Grazing	80 (20 + 20 + 20 + 20) 120 (30 + 30 + 30 + 30)	18	66 (33 + 33)

The stubble layer consists of plants remaining after grazing and cutting to the height of 5 cm above ground. The following assessments were made in the sampled soil material: granular size composition by sieve method, 1 mol dm^{-3} KCl by potentiometer, organic carbon by Tiurin method modified by Oleksynowa, total nitrogen by Kjeldahl method using Kjeltec apparatus, available P and K by Egner-Rhiem AL-method, available Mg by atomic absorption spectrometry AAS after extraction in 0.0125 mol CaCl$_2$ dm^{-3}. Obtained results were verified statistically by means of ANOVA using Statistica 6.0 application. Significance of differences was verified by means of Tukey's test on the confidence level of 0.05.

Results and discussion

After the four–year period of management and fertilization of the experimental treatments, an increase of P and K content by a decrease of assimilable Mg was registered in soils of all treatments (Table 2).

Table 2. Some properties of soil analysis

Variant	pH in KCl	Org. matter C	Total N	P	K	Mg
		g kg^{-1} soil			mg kg^{-1} soil	
Initial state	4.1	24.7	2.6	18	61	42
State after 4 years			Experiment I			
Control	4.2	20.8	3.4	9	52	40
N$_{80}$P$_{18}$K$_{66}$	4.0	17.3	4.0	15	49	36
N$_{120}$P$_{18}$K$_{66}$	3.9	18.5	3.7	12	43	32
LSD $_{0.05}$	n.s.	1.3	n.s.	3	6	7
Experiment II						
Control	4.1	25.0	3.1	10	46	40
N$_{80}$P$_{18}$K$_{66}$	4.1	17.2	4.0	12	58	31
N$_{120}$P$_{18}$K$_{66}$	4.2	18.7	3.5	16	60	37
LSD $_{0.05}$	n.s.	2.8	n.s.	4	9	5

n.s. – not significant

A)
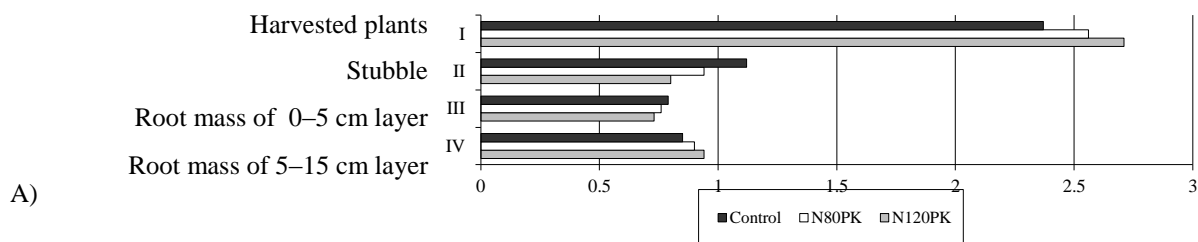
Variability coefficients: I) V–7% II) V–17% III) V–4% IV) V–5%

B)
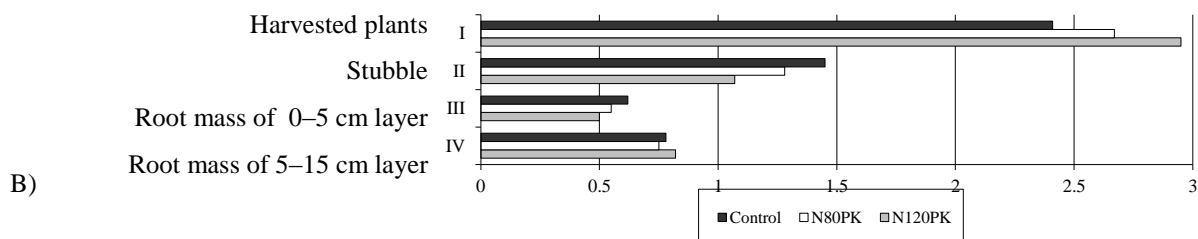
Variability coefficients: I) V–10% II) V–15% III) V–11% IV) V–4%

Figure 1. Mean content of Mg (g kg^{-1}) in the dry mass of the meadow and pasture flora in layers (A: Experiment I; B: Experiment II).

In the case of Mg this negative phenomenon may result from the more intensive utilization and, at the same time, greater soil exhaustion of available Mg. Grygierzec *et al*. (2007) found similar dependencies. The method of management and level of nitrogen fertilization affected Mg content in the plant material (Figure 1). Harvested plant mass was most abundant in this macroelement. Under cutting and pasture management the Mg concentration in harvested plants increased with the increase in rate of fertilization. Similar quantities of Mg in whole plant material might have been influenced by its availability. The experiments were located on soil that was initially poor in potassium. As reported by Barszczewski and Ducka (2012), potassium and magnesium antagonism in uptake by grassland plants was not registered despite the fertilization with potassium. According to Sapek (2008) acidity of soils may significantly alter Mg uptake by meadow plants.

Conclusions

After a four-year period of utilization, lower content of assimilable magnesium was found in the soil in relation with its initial state. Harvested plants were the most abundant in magnesium, whereas a shallower root mass revealed the least Mg quantities. Nitrogen fertilization increased the content of magnesium in harvested plants, while it decreased in the stubble and in root mass of the 0–5 cm layer in both experiments. The content of magnesium detected in the root mass of the 0–5 cm and 5–15 cm layers under pasture management was lower than in the case of combined utilization (hay production and grazing).

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Effects of previous cropping and establishment method on mineral concentration of whole-plant spring wheat

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Abstract

An experiment tested the null hypothesis that previous cropping and establishment methods would alter the mineral concentration of whole-plant spring wheat (WPSW) (*Triticum aestivum*). Four replicate plots (12 × 7.5 m) of perennial ryegrass (*Lolium perenne*), chicory (Ch) (*Cichorium intybus*), red clover (RC) (*Trifolium pratense*) and white clover (WC) (*Trifolium repens*) forages were established as pure swards in June 2009 in a randomized block design. Forages were harvested 5 times per annum during 2010-2012 and forage removed. Forages were sprayed using a non-selective herbicide before each plot was split, and spring wheat was sown in April 2013 either after ploughing or by direct drilling. Whole-plant spring wheat was sampled on 18 June for chemical analysis. Results showed previous cropping affected WPSW P, Mn and Cu concentrations. Lower N, P, K, S and Cu concentrations were found in WPSW after ploughing when compared with direct drilling. Within direct-drilled plots, WPSW following RC had higher Ca concentrations compared to other previous forage treatments. Within ploughed plots, WPSW following RC had a lower Mg concentration than Ch and WC. Overall, findings showed previous crop and establishment method alter mineral composition of WPSW but results varied for each mineral.

Keywords: spring wheat, chicory, minerals, trace elements, direct drill

Introduction

Some agricultural forages contain higher concentrations of minerals than ryegrass (Fisher and Baker, 1996) which may help to develop sustainable approaches to the management of these nutrients within agricultural systems. For example, chicory (*Cichorium intybus*) contains higher mineral and trace element concentrations than ryegrass (Marley *et al.,* 2013a;b). There has been extensive work on the effects of previous legume crops on subsequent cereal crops but much of this research focused on N utilization. Cultivation is known to change the availability of nutrients within soil, with previous research showing differences amongst cultivation techniques in their effects (Canell *et al.,* 1980). However, there has been little work into the effects of these previous crops on a subsequent cereal crop with regards to other essential minerals. Here, an experiment tested the null hypothesis that previous cropping and establishment methods would alter the mineral composition of spring wheat.

Materials and methods

Four replicate plots (12 × 7.5 m) of perennial ryegrass (PRG) (*Lolium perenne*) (cv. Premium), chicory (Ch) (*Cichorium intybus*) (cv. Puna II), red clover (RC) (*Trifolium pratense*) (cv. Merviot) and white clover (WC) (*Trifolium repens*) (cv. Aberdai) were established on 29 June 2009, in a randomized block design. PRG and Ch plots received inorganic N at 200 kg N ha^{-1} year^{-1}. During the harvest years 1-3 (2010-12), plots were harvested on 5 occasions per annum and evaluated as described in Marley *et al.* (2013a;b). In February 2013, Gallup 360 herbicide (360g l^{-1} glyphosate; Barclay Ltd, Dublin, Ireland) was applied at 4 l ha^{-1}. Soil was sampled per plot to a depth of 150 mm during March. Each plot was then split and allocated at random to one of two cultivation treatments. One half of each plot (3.75 m wide) was ploughed to a depth of 175 mm on 20 March and power-harrowed on 4 April (Ploughed). The other half plot

was left undisturbed and used for direct drilling (DD). On 5 April, spring wheat (*Triticum aestivum*) (cv. Tybalt) was sown on all plots using a Duncan Ecoseeder (Duncan Ag, Timaru, NZ) at a rate of 253 kg ha^{-1}, calculated to sow 569 viable seeds m^{-2}. All plots were flat rolled. Fertilizer was placed with the seed at sowing (49 kg N, 9 kg P_2O_5, 28kg K_2O and 16kg SO_3 ha^{-1}). Prilled lime was top dressed at 370 kg ha^{-1}. On 21 May (wheat at growth stage (GS) 25), fertiliser was applied at 127 kg N, 22 kg P_2O_5, 72 kg K_2O and 42 kg SO_3 ha^{-1}. On 18 June (wheat GS 32), samples of whole wheat plants were cut at 5 cm above ground level at 8 sites on each sub plot. Samples were oven dried, milled and submitted for chemical analysis by Inductively Coupled Plasma-Optical Emission Spectroscopy. N and S were determined by the Dumas Technique. Data were analysed by ANOVA as a split plot using Genstat® 11.1. Multiple comparisons were based on Bonferroni adjusted LSDs.

Results and discussion

Soil samples (data not shown) prior to wheat establishment showed no effect of previous cropping on pH or ammonium-N, P, Ca, Mg, B, Fe, Cu or Zn concentrations. Soil nitrate-N and K concentrations in PRG treatment were lower in other forage treatments ($P < 0.001$ and $P < 0.05$, respectively). Soil Mn concentration was higher where PRG was grown compared to other forages ($P < 0.05$). Results showed previous crop affected whole-plant spring-wheat P, Mn and Cu concentrations (Table 1). Lower N, P, K, S and Cu concentrations were found in WPSW after ploughing, compared with DD. Within DD plots, WPSW following RC had higher Ca concentrations compared with other previous forage treatments. Within ploughed plots, WPSW following RC had a lower Mg concentration than did Ch and WC. The Mn concentration of WPSW following Ch was higher than following WC. Higher Cu concentrations were observed following PRG compared with RC and WC, and lower following RC than both PRG and Ch. Within establishment method, Ca concentration was higher in RC than all other previous forage treatments. Within the ploughed treatment, wheat following WC had a lower Mg concentration than Ch and WC. No differences were observed between treatments in Fe, Zn or B concentrations (means: 56.2, 44.18, 3.21 mg kg^{-1} DM,).

Conclusions

Overall, findings showed previous cropping and establishment method alter the mineral composition of WPSW but results varied for each mineral.

Acknowledgements

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Table 1. Chemical composition (g/kg DM unless otherwise stated) of whole plant spring wheat established by ploughing or direct drill cultivation methods following pure swards of perennial ryegrass, chicory, red clover or white clover

		Previous Crop					s.e.m.		Prob		
	Establish.	Perennial Ryegrass	Chicory	Red Clover	White Clover	*Mean*	Rows	Columns	Previous Crop	Establish. Method	Prev.Est
Nitrogen	Conven.	35.47	37.25	33.12	35.32	*35.29*	1.734	1.377	0.065	<0.001	0.709
	D. Drill	39.85	39.97	35.32	38.07	*38.31*	16.6df	12df			
	Mean	*37.66*	*38.61*	*34.22*	*36.70*						
Phosphorus	Conven.	3.085	2.925	2.622	2.770	*2.851*	0.1329	0.1112	0.001	0.022	0.230
	D. Drill	3.405	3.130	2.635	2.817	*2.997*	17.5df	12df			
	Mean	*3.245^c*	*3.027^{bc}*	*2.629^a*	*2.794^{ab}*						
Potassium	Conven.	29.30	32.20	31.63	29.28	*30.60*	1.996	1.520	0.460	<0.001	0.101
	D. Drill	35.33	36.03	31.90	33.90	*34.29*	15.9df	12df			
	Mean	*32.31*	*34.11*	*31.76*	*31.59*						
Sulphur	Conven.	2.630	2.825	2.482	2.658	*2.649*	0.1397	0.1219	0.171	<0.001	0.500
	D. Drill	3.065	2.995	2.815	3.015	*2.972*	18.3df	12df			
	Mean	*2.847*	*2.910*	*2.649*	*2.836*						
Calcium	Conven.	3.260^a	3.250^a	3.905^b	3.420^a	*3.459*	0.1828	0.0775	0.055	0.604	0.030
	D. Drill	3.427^a	3.388^a	3.740^b	3.363^a	*3.479*	10.8df	12df			
	Mean	*3.344*	*3.319*	*3.823*	*3.391*						
Magnesium	Conven.	1.805^{ab}	1.917^b	1.640^a	2.062^b	*1.856*	0.1114	0.0718	0.095	1.151	0.026
	D. Drill	1.737	1.925	1.737	1.805	*1.801*	13.6df	12df			
	Mean	*1.771*	*1.921*	*1.689*	*1.934*						
Manganese (mg/kg DM)	Conven.	47.25	53.50	54.75	42.25	*49.44*	3.812	3.723	0.039	0.974	0.113
	D. Drill	50.50	54.75	46.25	46.50	*49.50*	20.3df	12df			
	Mean	*48.88^{ab}*	*54.12^b*	*50.50^{ab}*	*44.38^a*						
Copper (mg/kg DM)	Conven.	7.575	7.525	6.275	6.775	*7.037*	0.3630	0.3104	0.001	<0.001	0.197
	D. Drill	9.325	8.400	7.175	7.750	*8.162*	17.9df	12df			
	Mean	*8.450^c*	*7.962^{bc}*	*6.725^a*	*7.262^{ab}*						

Within rows, treatment values with differing lower case superscript differ significantly ($P < 0.05$)

Effect of soil amendment in the cultivation of selected grass species

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Abstract

Cultivation of *Dactylis glomerata* and *Festulolium braunii* was carried out in the years 2012-13. The following experimental treatments were applied: K- control (without fertilization and soil amendment), NPK - mineral fertilization, UG - UGmax preparation, EU - Ekoużyźniacz preparation, HA - Humus Active preparation, NPK + UG, NPK + EU and NPK + HA. Preparations were applied as sprays in both study years for the first regrowth of grasses. The following mineral fertilizations were used: N - 150 kg ha^{-1}, P - 80 kg P_2O_5 ha^{-1}, K - 120 kg K_2O ha^{-1}. All treatments were applied three times per year. Characteristics such as dry matter yield of plants and content of fibre fractions like NDF, ADF and ADL. The results were evaluated statistically by analysis of variance. Differentiations were verified by Tukey's test at a significance level $P \leq 0.05$. Regardless of the mineral fertilizers, soil preparations resulted in an increase in yield of *D. glomerata* and *F. braunii*. The studies showed that, regardless of the combination of fertilizer, the average annual yields of *D. glomerata* were over 17% higher than the yields obtained from *Festulolium braunii*. Fibre fractions, like NDF, ADF and ADL in the analysed plant material were not statistically differentiated and the values obtained were typical for the studied grass species.

Keywords: Humus Active UGax, mineral fertilization, grasses

Introduction

Soil fertilizer, due to the organic components such as humus and microorganisms, helps to improve the biological activity of the soil, increases the binding of free nitrogen from the air, and reduces erosion and losses of nutrients (Sosnowski and Jankowski, 2012). The formulations have also been attributed to the increased activation of the mineralization processes of organic components of soil (Sosnowski and Jankowski, 2013). The aim of the study was to determine the effect of three soil-improvement preparations, used against a background of NPK fertilization, on the yield and fibre fractions (NDF, ADF and ADL) in the dry matter of *Dactylis glomerata* and *Festulolium braunii*.

Material and methods

In the years 2012-13 cultivation of *Dactylis glomerata* L. cv. Sulino Bora and *Festulolium braunii* cv. was carried. Plot area was 6 m^2. The following experimental factors were used: K-control (without fertilization and soil fertilizer), NPK - mineral fertilization, UG - UGmax preparation, EU - Ekoużyźniacz preparation, HA - Humus Active preparation, NPK + UG , NPK and EU+ NPK+HA. Preparations were applied as sprays at dose rates of 0.9 litre ha^{-1} of preparation diluted in 350 litres of water. It was used in both years of the study for the first regrowth (phase of shooting). The composition of each formulation is shown in Table 1. Mineral fertilization rates were as follows: N-150 kg ha^{-1}, P-80 kg P_2O_5, K-120 kg K_2Oha^{-1}. All fertilizations were applied three times. Evaluations were made of the dry matter yield of plants and fibre fractions (NDF, ADF and ADL). Determination of the fractions was performed by NIRS method on NIRFlex N -500 using ready-calibration for dried fodder of the INGOT company. The results were evaluated statistically by analysis of variance. Mean differentiation was verified by Tukey's test at a significance level of $P \leq 0.05$.

Table 1. The composition of fertilizers

Preparation	Macroelements [g kg⁻¹]						Microelements [mg kg⁻¹]			
	N	P_2O_5	K_2O	Ca	Mg	Na	Mn	Fe	Zn	Cu
UG	1.2	0.5	3.5	-	0.1	0.2	0.3	-	-	-
EU	0.6	0.6	0.8	-	-	-	-	-	-	-
HA	0.2	3.0	5.5	3.0	0.5	-	15	500	3	1
Organic components										
UG	Lactic acid bacteria, photosynthetic bacteria, Azotobacter, Pseudomonas, yeasts, actinomycetes									
EU	Endomycorrhizal fungi, bacteria, enzymes involved in the metabolism of earthworms									

Results and discussion

Applied experimental factors: there were significant differences in the annual DM yields of crops (Table 2). Regardless of the species, on the treatments without fertilization, the highest yield (average 11.5 t DM ha⁻¹) occurred on treatments with Active Humus preparation. In contrast, the use of mineral fertilizers contributed to a significant increase in yield (13.1 t DM ha⁻¹) of plants supplied with UGmax (a combination of NPK + UG). It is worth noting that, regardless of the applied fertilizer combinations, the greater yields were obtained from *D. glomerata*: its average annual yields were over 11 t DM ha⁻¹and were 17.8% higher than the average annual yields of *Festulolium braunii.*

Table 2. Annual yield (t DM ha⁻¹) of *Dactylis glomerata* and *Festulolium braunii* depending on NPK and fertilizer type (average for research years). Means in rows marked with the same small letters do not differ significantly; means in columns marked with the same capital letters do not differ significantly.

Species	NPK fertilization										Mean
	No NPK				NPK				No NPK	NPK	
	K₀	UG	EU	HA	NPK	UG	EU	HA			
Dg	9.30 Ab	11.2 Aa	9.63 Ab	11.6 Ab	12.0 Ab	15.9 Aa	10.1 Ac	11.3 Bbc	11.6 Aa	11.2 Aa	11.4 A
Fb	5.96 Bc	9.19 Bab	8.49 Bb	11.5 Aa	9.51 Bbc	10.2 Bb	8.74 Bc	13.5 Aa	9.04 Bb	10.3 Ba	9.67 B
Mean	7.63 c	10.2 a	9.06 b	11.5 a	10.8 b	13.1 a	9.44 b	12.4 ab	10.3 a	108 a	

The data presented in Table 3 showed that, regardless of fertilization, in the cultivation of *D. glomerata* the highest production results were obtained using soil fertilizer UGmax.

Table 3. Annual yield (t DM ha⁻¹) *Dactylis glomerata* and *Festulolium braunii* depending on the applied fertilizer (average for research years. Means in rows marked with the same small letters do not differ significantly. means in columns marked with the same capital letters do not differ significantly.

Species	Fertilizer			
	K	UG	EU	HA
Dg	10.6 Ab	13.6 Aa	9.87 Ab	11.5 Bab
Fb	7.74 Bc	9.70 Bb	8.62 Bbc	12.5 Aa
Mean	9.17 b	11.7 ab	9.25 b	

The average annual yield on these treatments amounted 13.6 t DM ha^{-1} and was about 28% higher than the yields from the control. The use of Eco-fertilizer decreased average yields of *D. glomerata* in relation to the control by more than 7%. In turn, in *Festulolium braunii*, the highest efficiency was with the Humus Active preparation. Plants sprayed with this preparation contributed to yield increases from 7.74 (control) to 12.5 t DM ha^{-1} (for treatments receiving Humus Active).

Factors used in the experiment did not result in any statistically significant differences in contents of NDF, ADF and ADL fractions (Table 4).

Table 4. Content [% DM] of factions in dry matter of *Dactylis glomerata* and *Festulolium braunii* depending on the applied mineral fertilizers and soil fertilizer (average of from cuts and study years). Means in rows marked with the same small letters do not differ significantly; means in columns marked with the same capital letters do not differ significantly.

Species	NPK fertilization								Without NPK	NPK	Mean
	No NPK				NPK						
	K$_0$	UG	EU	HA	NPK	UG	EU	HA			
Neutral detergent fibre NDF											
Dg	52.7 Aa	54.4 Aa	57.2 Aa	50.1 Aa	52.4 Aa	54.1 Aa	48.6 Ab	54.1 Aa	53.6 Aa	52.3 Aa	53.0 A
Fb	48.6 Aa	44.3 Ba	48.7 Ba	51.7 A	52.7 Aa	52.1 Aa	54.0 Aa	52.9 Aa	48.3 Aa	52.9 Aa	50.6 A
Mean	50.7 a	49.4 a	52.9 a	50.9 a	52.6 a	53.1 a	51.3 a	53.5 a	50.9 a	52.6 a	
Acid detergent fibre ADF											
Dg	35.4 Aa	33.8 Aa	35.5 Aa	35.3 Aa	29.7 Aa	30.8 Aa	30.9 Aa	31.3 Aa	35.0 Aa	30.7 Aa	32.9 A
Fb	35.8 Aa	34.7 Aa	33.5 Aa	35.6 Aa	30.8 Aa	28.2 Aa	30.5 Aa	30.6 Aa	34.4 Aa	30.0 Aa	32.2 A
Mean	35.7 a	34.3 a	34.5 a	35.5 a	30.3 a	29.5 a	30.7 a	31.0 a	34.7 a	30.4 a	
Acid detergent lignin ADL											
Dg	4.25 Aa	3.54 Aa	3.65 Aa	4.22 Aa	4.43 Aa	4.50 Aa	4.61 Aa	4.13 Aa	3.92 Aa	4.42 Aa	4.17 A
Fb	3.79 Aa	4.35 Aa	4.27 Aa	4.45 Aa	4.45 Aa	4.47 Aa	4.64 Aa	4.45 Aa	4.22 Aa	4.50 Aa	4.36 A
Mean	4.02 a	3.95 a	3.96 a	4.34 a	4.44 a	4.49 a	4.63 a	4.29 a	4.07 a	4.46 a	

In previous studies (Sosnowski and Jankowski 2012) there were also no differences in the proportion of fibre fractions in the dry matter of grasses grown in pure stands on arable land

Conclusion

Regardless of the fertilization, the use of soil improvement preparations resulted in an increase in yield of *D. glomerata* and *Festulolium braunii*. The best production of *D. glomerata* was obtained by using the soil fertilizer UGmax, but for *Festulolium braunii* this was with Humus Active preparation. The application of NPK resulted in a 29% increase in crop yield when sprayed with UGmax. The studies have shown that, regardless of the combination of fertilizer, the average annual yields of *D. glomerata* were over 17% higher than the yields obtained from

Festulolium braunii. Fibre fractions, like NDF, ADF and ADL in the analysed plant material were not statistically differentiated and the values obtained were typical for the studied grass species.

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Milk production and profitability in relation to size of grassland farms

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Abstract

In recent years the most profitable activity in agriculture is considered to be with milk production. The effectiveness of milk production depends primarily on the direct costs and the obtained price. Therefore the aim of this work was to analyse the financial results of dairy farms in the eastern part of the Mazovia region. The research was completed in 2010. The owners of 36 farms were sent a questionnaire containing 18 questions. The whole population was devoted to six production groups, depending on the number of physical units of dairy cows. Based on survey data, an analysis of the profitability of milk production was done, and statistical analysis by calculating the Pearson linear correlation coefficient (r) and coefficient of determination (R^2) was done. The study showed that the highest annual yield of milk from a cow at the level of 7500 kg, was obtained by farmers with an area of 19.7 ha, with the density of 1.82 BFU ha^{-1}. The performed regression analysis showed significant positive correlation between the effectiveness index and the surface agricultural lands in the farms or density of agricultural lands. Milk production was profitable on farms that had suitable areas of grasslands.

Keywords: grassland, milk production, farms, costs

Introduction

In Poland, milk production has had the largest share of commercial agricultural production over many years, and the dairy sector (production and processing) involves 25% of the industry workforce and produces 17% of total production of the agricultural industry. Therefore, the milk market is one of the basic agri-food markets in our country. In addition, it is worth noting, that in 2008, Polish exports of dairy products on to European markets increased by up 73.6% (Baer-Nawrocka and Kiryluk-Dryjska, 2010). It can therefore be assumed that the Polish dairy sector is gaining importance in western markets.

The aim of the study was to analyse of the financial results of farms producing milk, taking into account the factors of production occurring within farm, and market conditions affecting the profitability of the business.

Material and methods

Research by direct interview was conducted in 2010 on 36 farms from the eastern Mazovia region. A questionnaire containing 18 questions was sent to farm owners. The obtained data were used to characterize the various research objectives, resulting in a breakdown of the analysed population into 6 groups, depending on the number of physical units of dairy cows: group A up to 10, B - from 11 to 15, C - from 16 to 20, D - from 21 to 25, E - from 26 to 30, F - over 30. Moreover, in the study population, farms from group A, having 10 dairy cows, dominated. In the other production groups there were from 5 to 6 dairy farms. On the basis of questionnaire data, an analysis taking into account the profitability of milk production has done with the following economic size: the cost of herd renewal, the cost of feed (e.g. feed purchased), own feed, own meadow hay; own corn silage, own mineral mixture and the cost of specialist. Profitability index as the value of effectiveness ratio was calculated for each frm and are presented as average from production group, according to the following formula (Kisiel *et al.,* 1996): Wo = (Wp : Wk) × 100%. where: Wo - value of effectiveness ratio, Wp - value of commodity production, Wk - value of direct costs. In addition, statistical analysis was performed by calculating the Pearson correlation coefficient (r) and coefficient of determination

(R^2) between the effectiveness ratio and the following variables: density of GPU ha^{-1} of arable lands, surface of arable lands in hectares, the average annual milk yield of cows in kg, the price of 1 litre of milk in PLZ.

Results

The obtained data show that in farms with a higher number of cows, milk yield was two times higher than on farms with up to 10 dairy cows. The highest value of this parameter (7500 kg milk per year per cow) was achieved in farms with an average of 28 dairy cows (group E), used during a period of 8 years, with an average of approximately 19 ha of arable land and about 14 hectares of meadows and pastures. The study also showed a significant positive correlation between the surface of agricultural land and the density of dairy cows and the profitability of milk production (Table 1).

Table 1. Statistical analysis for relation between individual diagnostic features

	r	R^2	y = ax ± b	S_{yx}	$S_{yx\%}$
index of milk production profitability x surface of agricultural lands in studded farms	0.624	0.39	y = 121.96 + 1.223x	275	3.7
index of milk production profitability x milk cows density on agricultural lands	0.758	0.59	y = 112.63 + 36.471x	1.86	2.4
index of milk production profitability x mean annual yield	0.989	0.98	y = 87.694 + 0.1205x	1.29	1.6
index of milk production profitability x value of milk price	0.655	0.43	y = 12.092 + 103.48x	2.35	3.1

r -correlation coefficient, R^2 –determination coefficient S_{yx} –standard deviation ; $S_{yx\%}$– relative estimate error

The correlation coefficient for this relationship was 0.624 and 0.758 respectively. Furthermore, data suggest that the profitability of the analysed production was largely influenced by the average annual yield of milk per cow. This feature, up to 98 % (R^2 = 98 %) determined the volatility of the effectiveness ratio achieved in this population farms. In addition, there was wide diversity in milk prices. The highest unit prices were negotiated farmers with more than 26 dairy cows - Groups E and F. On average, these farms were paid from 1.45 to 1.54 PLZ per litre.

Economic analysis of farms producing milk plays an important role. The data in Table 2 show that the value of commodity production within the analysed groups varied widely and ranged from 4560 PLZ (in farms from group A) to 11935 PLZ (in farms from group E). A similar tendency was also analysed in the direct costs of production. Farms with fewer cows also had the lowest costs in their business (3474.70 zł - Group A), while for farms that had more than 25 cows the value of direct costs were higher by more than 1,300 zł. Average gross margin per cow, obtained by the average farm producing milk in the study area, was 2.148 PLZ. In contrast, in analysing the value of the surplus, which reached the farms from various production teams, great diversity can be seen. The highest value of surplus was obtained farmers having an average of 26-30 dairy cows (3810 PLZ - Group E), and in second place were the farmers that had more than 30 cows (2550.33 PLZ - Group F). In terms of milk production, the least income was earned by the farmers having up to 15 dairy cows (528.00 PLZ - Group B). A similar tendency was observed in terms of the breakdown of the profitability index of milk production.

Table 2. Analysis of financial results in studied farms by production groups (mean from farms)

Production groups	Value of commodity production (receipts) [zł]	Direct cost [zł]	Direct surplus [zł]	Index of milk production profitability [%]
A	4066.60	3049.95	1016.65	133.33
B	2400.00	1872.00	528.00	128.20
C	3416.66	2425.36	991.30	140.87
D	3995.83	2797.08	1198.75	142.85
E	7700.00	3890.00	3810.00	197.94
F	5931.00	3380.67	2550.33	175.43
Mean	4585.01	2902.51	2148.68	157.96

Conclusion

The highest milk yield per cow was at the level 7500 kg per year, which was reached by farmers with an area of arable land of 19.7 ha. Annual gross margin for agricultural farms producing milk per cow ranged from 528 to 3810 PLZ. There was a significant impact on this value of the surface area of arable land, cow density, the average annual milk yield per cow and the unit price of milk. The development of dairy farms and the economic benefits derived from this activity are influenced by many factors. The most important of them are efficiency, which most determines the profitability of milk production (98%) and quality of produced raw material, which affected the value of the obtained purchase prices.

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Meadow apophytes in segetal communities

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Abstract

The work analyses the share of meadow apophytes in the structures of segetal communities. Floristic studies were conducted from 1995 to 2011 in east-central Poland. A total of 85 species representing meadow apophytes were found in winter and spring cereals as well as tuber and root crops, very rare and rare species being the most numerous group. The group of frequent and common apophytes comprised 14 species. Frequency of occurrence and the coverage of these species are dependent on the agricultural technology.

Keywords: meadow apophytes, semi-natural and anthropogenic habitats, adaptation, biodiversity

Introduction

One of symptoms of flora synanthropisation is invasion of native species from natural and semi-natural habitats, such as meadow habitats, to disturbed habitats. The process does not markedly affect the qualitative changes of flora but it indicates the adaptive attributes that some species have. Due to changes that have recently taken place in the cropping profile, as well as methods and means of agricultural production, agrocenoses are becoming more and more floristically impoverished. At the same time, the compensation of ubiquitous species is increasing thus destroying structures of segetal communities (Stehlik *et al.,* 2007, Storkey *et al.,* 2011). Native species spreading from meadow habitats increase the floristic diversity of segetal communities (Skrajna *et al.*, 2010; Dąbkowska and Sygulska, 2013). The objective of this work was to: indicate apophytes in the flora of segetal communities in east-central Poland; determine, using the frequency of occurrence, constancy of occurrence and the index of coverage, the role that meadow apophytes play in agrocenoses; indicate the meadow species that are best adapted to occur in segetal communities.

Materials and methods

Floristic studies were carried out from 1995 to 2011 in east-central Poland. The area is part the Central Mazovian Lowland and it covers over 20 thousand km^2 (Kondracki, 2009). The dominant soils are podzols and, in valley bottoms, alluvial soils of various origins. Locally on denudating plains, chernozems developed on periglacial silty and clay landforms. Places located lower are surrounded by bog and post-bog soils.

The present work is based on over 1200 floristic inventories which were performed in cereal, root and tuber crops as well as stubbles. Next, meadow apophytes, which established in the agrocenoses as secondary habitats, were determined in the flora of these communities. Apophyte occurrences in field habitats were characterized by describing the following: soil conditions, frequency and constancy of occurrence, and the index of coverage in various habitats where they were found.

Results

Agrocenoses in east-central Poland comprise 85 meadow apophytes which made the segetal flora in this area richer (Table 1). Analysis of the biological spectrum revealed that hemicryptophytes were the most numerous group (60 species), followed by geophytes (14 species) and therophytes (11 species). Meadow apophytes occurred in winter and spring cereals and non-cultivated stubble fields. They also established in dense stands of tuber and root crops. Some of the species remained in communities occupying edges of fields and field borders. Very rare and rare species formed the richest group (71) and included, e.g.: *Bromus hordeaceus, Alopecurus geniculatus, Plantago media, Phleum pratense, Lathyrus pratensis, Rumex confertus, Knautia arvensis*. This group was followed by common and frequent meadow apophytes (14), e.g.: *Achillea millefolium, Artemisia vulgaris, Cerastium holosteoides, Plantago lanceolata, Equisetum arvense*. Meadow apophytes were found in segetal communities established on various soil types and kinds (Table 1), for example: grey brown podzolic soils which formed from sands of various origins, silty soils, true and degraded charnozems, brown soils and alluvial soils.

Table 1. Meadow apophytes in the agrocenoses of east-central Poland; common and frequent species

S.n.	Species	Life form	Winter cereals	Spring cereals	Tuber crops	Stubble-fild
1.	*Achillea millefolium* L.	H	A,Bw,Dz,F ps;pgl;pgm;gl.płz;płi pH – 5.5-8.0 S=I-II;W=4-37	A,Bw,Dz,F psp;pgl;pgm;płz;płi pH – 4.5-7.5 S=I;W=7-20	A, Bw,Dz,F ps;pgl;pgm;płz pH – 4.5-6.0 S=I-II ;W=3-30	A,Bw,Dz,F ps;pgl;pgm;płz;płi pH – 4.5-8.0 S=II-V;W=7-195
2.	*Artemisia vulgaris* L.	H	A,Bw,Dz,F ps;pgl;pgm;gl;płz pH – 4.5-8.0 S=I-II;W=8-47	Bw,Dz,F psp;pgl;pgm;płz;gs pH – 4.5-7.5 S=I;W=5	A, Bw,Dz,F ps;pgl;pgm;płz;płi pH – 5.0-6.5 S=I-II;W=8-39	A,Bw,Dz,F ps;pgl;pgm;gsp.płz pH – 4.5-8.0 S=II-IV;W=9-61
3.	*Cerastium holosteoides* Fr.em.Hyl.	H	A,Bw,Dz,F psp;pgl;pgm;płz pH – 5.5-7.5 S=II-IV;W=12-37	Bw,Dz,F pgl;glp;pgm;płz pH – 4.5-7.5 S=I;W=3-7	Bw,Dz,F pgl;pgm;gsp;płz pH – 6.0-7.5 S=I;W=5-20	Bw,Dz,F psp;pgl;glp;pgm;płz pH – 4.5-7.5 S=II-IV;W=20-100
4.	*Equisetum arvense* L.	G	A,Bw,Dz,F ps;pgl;pgm;gl;płz pH – 4.5-7.0 S=II-IV;W=31-160	Bw,Dz,F psp;pgl;pgm;gs;płz pH – 4.5-6.5 S=II-IV;W=30-173	A, Bw,Dz,F ps,pgl;płz pH – 4.5-6.0 S=II-V ;W=50-417	A,Bw,Dz,F ps;pgl;pgm;gl;płz pH – 4.5-6.5 S=II-V;W=30-305
5.	*Glechoma hederacea* L.	H	Bw,F pgl;płz pH – 5.0-7.0 S=I;W=5	Bw,Dz,F pgl;płz pH – 4.5-7.5 S=I;W=5	A,Bw, F ps;pgl;płz pH – 5.5-6.5 S=I-III;W=10-50	A,Bw,Dz,F pgl;pgm;płz pH – 5,0-7,5 S=I-III;W=30-65
6.	*Melandrium album* /Mill./ Garcke	T	A,Bw,Dz,F psp;pglp;gsp;płz pH – 5.0-7.0 S=II;W=5-20	Bw,Dz,F psp;pgl;płz; pH – 4.5-7.5 S=I;W=16	A, Bw,Dz,F ps;pgl;płz pH – 5.0-6.5 S=II;W=5-20	A,Bw,Dz,F psp;pgl;pgm;płz pH – 5.0-7.0 S=II-IV;W=21-465

			A,Bw,Dz,F	Bw,Dz,F	A, Bw,Dz,F	A,Bw,Dz,F
7.	*Poa annua* L.	T	ps;pgl;glp;płz pH – 4.5-7.5 S=I-II;W=3-10	psp;pgl;płz pH – 4.5-7.5 S=I-II;W=4-25	ps;pgl;płz pH – 5.0-6.5 S=I-IV;W=12-48	ps;pgl;pgm;płz pH – 4.5-7.5 S=II-IV;W=7-67
8.	*Plantago lanceolata* L.	H	A,Bw,Dz,F ps;pgl;płz pH – 5.5-7.0 S=I-II;W=25	Bw, F pgl;płz pH – 5.0-7.0 S=I;W=5	A, Bw,Dz,F ps;pgl;pgm;płz pH – 4.5-6.0 S=I-III;W=5-45	A,Bw,Dz,F ps;pgl;pgmp;płz pH – 4.5-7.5 S=I-IV;W=8-70
9.	*Potentilla anserina* L.	H	Bw,Dz,F pgl;pgm;płz pH – 4.5-7.0 S=II;W=5-25	Bw,Dz,F psp; płz pH – 6.0-7.0 S=I;W=5	A, Bw,Dz,F pgl;płz pH – 5.0-6.5 S=I-II;W=7-47	Bw,Dz,F pgl;pgm;płz pH – 4.5-7.0 S=I-III;W=5-50
10.	*Ranunculus repens* L.	H	Bw,Dz,F, pgl;płz;płi pH – 5.5-7.0 S=II-III;W=5-40	Bw,F pgl;płz pH – 6.0-7.0 S=I;W=3-15	Bw,Dz,F pgl;płz pH – 5.0-6.5 S=I-III;W=3-50	Bw,Dz,F pgl;płz; płi pH – 5.5-7.0 S=I-IV;W=15-65
11.	*Stachys palustris* L.	G	Bw,Dz,F pgl;pgm;płz;płi pH – 5.0-7.5 S=II-IV;W=31-123	Bw,Dz,F pgl:gl;płz pH – 4.5-7.5 S=I-III;W=4-84	Bw,Dz,F pgl;płz pH – 5.5-7.0 S=I-V;W=10-430	Bw,Dz,F pgl;pgm;gl;płz;płi pH – 4.5-7.5 S=I-V;W=20-715
12.	*Stellaria media* /L./ Vill.	T	Bw,D,Dz,F ps;pgl;pgm;płz pH – 5.0-7.0 S=I-IV;W=8-15	Bw,Dz,F pgl;pglp;pgm;płz pH – 4.5-7.5 S=I-III;W=38-138	Bw,Dz,F ps;pgl;płz pH – 4.5-6.5 S=II-V;W=75-438	Bw,Dz,F ps;pgl;pgm;płz pH – 4.5-7.5 S=I-IV;W=20-1245
13.	*Taraxacum sp.* F.H. Wigg. coll.	H	Bw,Dz,F ps;pgl;pgm;płz pH – 5.0-8.0 S=II-IV;W=8-47	Bw,Dz,F ps;pgl;płz pH – 4.5-7.5 S=I-II;W=3-25	A,Bw,Dz,F ps;pgl;płz pH – 5.0-6.0 S=I-IV;W=10-73	A,Bw,Dz,F ps;pgl;pgm;płz pH – 4.5-8.0 S=II-V;W=20-154
14.	*Trifolium repens* L.	H	A,Bw,Dz,F ps;pgl;pgm;płz pH – 5.5-7.0 S=II-IV;W=13-87	Bw,Dz,F ps; pgl;płz pH – 4.5-7.5 S=I-II;W=17-35	A,Bw,Dz,F ps;pgl;płz pH – 4.5-6.5 S=I-II;W=7-43	A,Bw,Dz,F ps;pgl;pgm;płz pH – 4.5-7.0 S=I-IV;W=10-86

Very rare: *Alchemilla monticola* Opiz – H, *Alopecurus geniculatus* L. – G, *Alopecurus pratensis* L. – G, *Anhriscus sylvestris* (L.) Hoffm. – H, *Arrhenantherum alatius* (L.) P. Baeur. ex J. Presl & C. Presl – H, *Bellis perennis* L. – H, *Briza media* L. - H, *Campanulla patula* Griseb. – H, *Carex hirta* L. - G, *Carum carvi* L. – T, *Crepis biennis* L. – T, *Festuca rubra* L. s.s – H, *Filipendula ulmaria* (L.) Maxim. – H, *Heracleum sibiricum* L. – H, *Heracleum sphondylium* L. S.STR. – H, *Hieracium umbellatum* L. – H, *Leontodon hispidus* L. – H, *Leucanthemum vulgare* Lam. S. STR – H, *Lichnis flos-cuculi* L. – H, *Luzula campestris* /L./ DC. – H, *Lythrum salicaria* L. – H, *Polygonum bistorta* L. – G, *Ranunculus acris* L. S. STR. – H, *Sanguisorba officinalis* L. – H, *Tragopogon pratensis* L. S. STR – H,

Rare: *Agrostis capillaris* L. – H, *Bromus hordeaceus* L. – H, *Centaurea jacea* L. – H, *Crepis capillaris* (L.) Wallr. – T, *Equisetum palustre* L. – G, *Festuca pratensis* Huds. – H, *Gagea pratensis* (Pers.) Dumort – G, *Galium mollugo* L. – G, *Galium verum* L. – G, *Geranium pratense* L. – H, *Holcus lanatus* L. – H, *Hypochoeris radicata* L. – H, *Lathyrus pratensis* L. – H, *Lysimachia nummularia* L. – G, *Lysimachia vulgaris* L. – G, *Odontites serotina* (Lam.) Rchb. – T, *Pastinaca sativa* L. s.s. – H, *Phleum pratense* L – H, *Plantago media* L. – H, *Potentilla reptans* L. – H, *Prunella vulgaris* L. – H, *Ranunculus sardous* Crantz – H, *Rumex acetosa* L. – H, *Rumex confertus* Willd. – H,

Trifolium campestre Schreb. – T, *Trifolium dubium* Sibth. – T, *Trifolium hybridum* L. – H, *Veronica chamaedrys* L. S. STR. – H, *Veronica serpyllifolia* L. – H,

Dość rzadkie: *Agrostis gigantea* Roth. – H, *Agrostis stolonifera* L. – H, *Centaurium pulchellum* (Sw.) Druce – T, *Dactylis glomerata* L. – H, *Equisetum sylvaticum* L. – G, *Hypericum perforatum* L. – H, *Knautia arvensis* /L./ J.M. Coult. – H, *Leontodon autumnalis* L. – H, *Lolium perenne* L. – H, *Lotus corniculatus* L. – H, *Poa pratensis* L. – H, *Poa trivialis* L. – H, *Rumex crispus* L. – G, *Sagina procumbens* L.- H, *Stellaria graminea* L. – H, *Trifolium pratense* L. – T, *Vicia cracca* L. – H,

Explanations: T-terophytes, H-hemicryptophytes, G-geophytes; S-constancy class; W-cover coefficient; A-podsolic soil; Bw- brown leached; F-alluvial, D- proper black earth, Dz-degraded black earth; ps-slightly loamy sand; pgl-light loamy sand; pglp-silty light loamy sand; pgmp-hefty dusty loamy sand; psp-silty slightly loamy sand; pgm-heavy loamy sand; płz-silt; płi- gl-light clay; gs-maen clay; glp- silt loam; gsp- mean clay silt

Conclusions

Meadow apophytes enrich the segetal flora of east-central Poland. Frequent or mass occurrence of these species is closely connected with conventional soil and crop plant cultivation. An introduction of new technologies into agriculture removes meadow apophytes from segetal communities.

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Effectiveness of grassland management and mechanical methods for the weed control of Colchicum autumnale in permanent meadows

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Abstract

Several measures are commonly suggested to control meadow saffron (*Colchicum autumnale* L.), a poisonous weed well adapted to extensive management, but clear experimental evidence is not available for all of them. For this reason, the effect of two mechanical methods (early cut, rolling with a spike roller) in combination with fertilization (present/absent) and oversowing (present/absent) was investigated in a four-year field trial at two mountain permanent meadows. An average efficacy of 15% in reducing plant density was found following three consecutive treatments with an early cut, while rolling had virtually no effect on it. An early cut resulted also in a rapid decrease over the years of plant fresh weight and the proportion of fertile plants of *Colchicum*. No effect was detected for fertilization and oversowing. In order to achieve a strong reduction of *Colchicum* density, an early cut needs to be applied over a long time.

Keywords: *Colchicum autumnale*; cut; spike roller; oversowing; fertilization

Introduction

Meadow saffron (*Colchicum autumnale* L.) is a toxic perennial geophyte with an unusual phenology, with flowering taking place in autumn and a short photosynthetically active period finishing in the middle of summer; for this reason, extensive management with late mowing or grazing promotes population growth and can lead to detrimental effects for forage quality and marketability of hay (Jung *et al.*, 2011, Winter *et al.*, 2011). Increasing abundance of *Colchicum autumnale* has been also repeatedly observed in the mountain region of South Tyrol. These reports apply mostly to permanent meadows with an unusually late first cut, which is mainly related to logistic issues of the farm management. Several measures concerning grassland management and mechanical weed control are reported by codes of practice (see, e.g., Caputa, 1984), but clear experimental evidence for the effectiveness of some of them is only partly available in the literature. In order to get reliable information for some of these methods (early cut, mechanical damage by means of a spike roller, also in combination with fertilization), a four-year field experiment was conducted in South Tyrol between 2009 and 2012. The effect of oversowing, a popular measure to improve forage composition, was investigated as well.

Materials and methods

A four-year field experiment was conducted in two permanent mountain meadows with a moderate infestation of *Colchicum autumnale* at an altitude around 1500 m a.s.l. (Table 1).

Table 1. Description of the experimental sites.

Experimental site	Geographic coordinates	Altitude (m a.s.l.)	Slope (%)	Exposition	*Colchicum*-density at trial start (plants m^{-2})	*Colchicum*-fresh weight at trial start (g m^{-2})
St. Veit	46° 43' 20" N 12° 43' 20" E	1490	60	S	5.6	65.2
Kameriot	46° 42' 6" N 12° 9' 44" E	1460	15	W	8.6	37.2

Both meadows are yearly cut twice, with the first cut being performed quite late (after mid June). Four factors were investigated: (i) weed control method: cut at ground level of *Colchicum*-plants by means of electric scissors (mowing), rolling using a motor mower for steep slopes (PS15, Brielmaier, Friedrichshafen, D), equipped with two 0.5 m-wide spike rollers instead of wheels and a machine weight of 200 kg, and an untreated control. Mechanical treatments were applied between beginning and completion of capsule formation, roughly corresponding to end of May-beginning of June; (ii) fertilization with 20 m³ ha^{-1} of 2:1 water-diluted slurry applied after mechanical weed control (present/absent); (iii) oversowing (present/absent) after mechanical weed control with 20 kg ha^{-1} of a seed mixture (12% weight *Agrostis tenuis*, 6% *Trisetum flavescens*, 12% *Dactylis glomerata*, 28% *Festuca rubra*, 17% *Festuca pratensis*, 5% *Lolium perenne*, 8% *Phleum pratense*, 12% *Poa pratensis*). After seeding, harrowing was simulated by manual raking of the plots. An orthogonal design was laid out as a randomized complete block design with three replicates and a plot size of 2 m² (1 × 2 m). In each plot, the number of *Colchicum*-shoots was counted before applying the weed control treatments. The fresh weight of the epigeal *Colchicum*-biomass and the number of generative plants with capsules were assessed in the mown plots. Treatment efficacy on shoot density was calculated with the methods of Henderson and Tilton (1955), which also takes in account the changes of plant density in the control plots to assess treatment efficacy. Negative efficacy values, corresponding to increases of plant density with respect to the control treatment, were allowed to occur up to -100%, while 12 implausible values were removed from the data set. Data were analysed by means of a mixed model assuming the weed control method (only for the effectiveness data), the fertilization, the oversowing and the year as well as their interactions to be fixed effects and accounting for serial correlations due to repeated measures of the factor year with the plot as a subject. The block effect, the site factor and the interactions of the latter with the fixed factors up to the second order were considered to be random effects. Multiple comparisons were performed by Sidak's test. The share of generative plants with capsules was analysed by means of non-parametric tests (Friedman test and multiple comparisons by Wilcoxon-Wilcox) because of violation of the assumptions of normal distribution of residuals and variance homogeneity even after data transformation. $P \leq 0.05$ was considered to be significant.

Results and discussion

Efficacy in reducing the density of *Colchicum* plants was found to be affected by the weed control method only ($P = 0.012$), whilst no effect of fertilization ($P = 0.69$) and oversowing ($P = 0.60$) and of their interactions with other factors was detected. More precisely, a marginal significance of the control method with the year was observed ($P = 0.50$), as the efficacy of an early cut was found to differ from that of the rolling treatment only in the last observation year (Table 2).

Table 2. Efficacy (%) of the weed control methods over the investigation period. Means without letters in common within each year significantly differ from each other.

Weed control method	Year			Average across all years
	2009-2010	2010-2011	2011-2012	
Early cut	16.5 [a]	-2.8 [a]	31.0 [a]	14.9 [a]
Spike roller	6.2 [a]	2.5 [a]	-6.3 [b]	0.8 [b]

On average after three treatment seasons, the use of a spike roller seemed to produce no relevant effects, while the early cut led to an efficacy of about 15%. These results support recent experimental evidence about the negative effect of an early cut on population growth of

Colchicum autumnale (Winter *et al.*, 2014), although the timing of the cut in the present experiment was not strictly bound to a well defined phenological stage (about 25 cm plant height and capsules with their top being about 10 cm above ground), as suggested by Jung *et al.* (2012). The mowing treatment resulted also in a decrease over time of the fresh weight of *Colchicum*, expressed as epigeal biomass as well as the mean plant weight and led to a very low proportion of fertile plants with capsules (Table 3).

Table 3. Changes over time of plant biomass and plant weight of *Colchicum autumnale* and of the proportion of generative plants with capsules by performing a yearly mowing treatment with an early cut.

Variable	Year			
	2009	2010	2011	2012
Colchicum biomass fresh weight (g m^{-2}) [#]	26.6 [a]	17.1 [b]	4.2 [c]	4.0 [c]
Colchicum plant fresh weight (g plant^{-1}) [#]	6.7 [a]	3.6 [b]	1.0 [d]	1.6 [c]
Proportion of plants with capsules (%) [†]	33.8 [a]	22.6 [a]	1.7 [b]	1.3 [b]

[#] Analysis with mixed model, logarithm-transformed data. Back-transformed means are shown
[†] Friedman-test and multiple comparisons by Wilcoxon-Wilcox-test

Whilst the lack of an effect of oversowing is not surprising because of to the known aleatory effect of this practice (Huguenin-Elie *et al.*, 2006) and of the only light disturbance to the existing vegetation provided by raking, the lack of evidence for an effect of the fertilization was less expected, as this advice is often mentioned in codes of practice in order to enhance competition on *Colchicum* by other forage species.

Conclusions

The results provide evidence of the effectiveness of an early cut on the control of *Colchicum autumnale* in permanent meadows. This treatment contributes to a rapid reduction in plant weight and in the proportion of fertile plants, but it needs to be applied over a long time to achieve a strong reduction of plant density. The effect of often recommended measures, such as fertilization or rolling with a spike roller, could not be confirmed by the outcome of the present experiment.

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Issues regarding the genus Fusarium in permanent grassland

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Abstract

The objective of this study was to detect *Fusarium* spp. in plant material and the air of permanent grasslands. The number of *Fusarium* colony-forming units (CFU) was significantly higher in the green mass during August than in May. The occurrence of *Fusarium* spp. was greater in the green mass at Lukov than at Závišice. In the air above the grasslands, the amount of *Fusarium* CFU was higher in August, but the differences were statistically non-significant.

Keywords: *Fusarium*, permanent grassland

Introduction

Permanent grasslands (hereafter PG), consisting of meadows and pastures, are part of the agricultural land resource along with arable land, hop fields, orchards and gardens. They have characteristic plant communities that are dominated by species of the Poaceae, often with significant representation of clover, and also with the presence of sedges, rushes, bulrushes and a number of other plants. In addition to their economic functions, PG have a great number of non-productive functions, such as in conserving the biological diversity and ecological stability of a region, protecting surface water and groundwater resources, protecting the soil, and others (Rychnovská, 2008).

Fusarium spp. are among the most serious contaminants of PG (regarding haylage produced from them), as these have allergenic effects on people as well as animals. They include significant producers of mycotoxins, in particular trichothecenes (deoxynivalenol, nivalenol, T-2 toxin), which can have immunosuppressant, oestrogenic and teratogenic effects. In addition to these effects, many *Fusarium* spp. cause a number of respiratory tract illnesses, such as chronic colds, asthma and other allergenic reactions.

Among the most commonly occurring diseases in grasses are pink snow mould, the source of which is the species *Microdochium nivale* (also known as *Fusarium nivale*), and Fusarium ear blight, caused by the species *F. culmorum*, *F. graminearum*, *F. poae* and *F. avenaceum*. The pathogens survive in the forms of mycelia or conidia in infected plants, soil, and dead plant matter. Many species also create chlamydospores, which aid in their survival under adverse conditions (Cagaš and Macháč, 2005; Smiley *et al.*, 2007).

Fusarium spp. are found in abundance not only in plant material of PG, but also in the air. During operations to maintain PG, the particles, spores, and hyphae of *Fusarium* spp. are released into the air and can induce mycoses (external and internal), allergic reactions, chronic colds and asthma. Species from the genera *Alternaria*, *Cladosporium* and *Penicillium* can also be found in the air. In addition, many of these fungi are among the producers of mycotoxins, which can have carcinogenic and mutagenic effects (Cvetnić and Pepeljnajk, 1997; Kakde and Kakde, 2012).

This work presents recent results from studies examining the presence of microscopic fungi in permanent grasslands with a focus on *Fusarium* spp. The presence of *Fusarium* spp. was monitored in plant material and in the air at two separate locations in the Czech Republic.

Materials and methods

During 2013, samples of green mass and air were taken twice (before the first and second cuttings, in May and August respectively) at two locations: Závišice (Nový Jičín District, 281 m a.s.l.) and Lukov (Zlín District, 334 m a.s.l.). A phytosociological survey was undertaken

during sample collection. The green mass was always collected into a sample mixed from 4 randomly selected places within the monitored PG and, at the same time, Petri dishes with potato dextrose agar (PDA) were exposed in order to capture spores from the air (one Petri dish per plot; exposure time 10 min). The plant material was cut into segments (2–5 mm), which were subsequently placed in Petri dishes containing PDA. Each sample was processed into six dishes with agar medium (10 segments per dish). At the same time, the dry matter of the plant mass in the sample was determined and the number of detected colony-forming units (CFU) calculated per gram of dry matter.

In addition to the detailed quantitative evaluation as to the presence of *Fusarium* spp., other genera of microscopic fungi were determined morphologically in the samples. Isolated *Fusarium* spp. were preserved in a collection of cultures for subsequent species identification, which is still ongoing. The collected data were evaluated statistically using the Statistica 10 program. Analysis of variance (ANOVA) was run, along with Cochran's and Bartlett's tests to verify homogeneity of variance, and, in cases of non-homogeneity of variances, Box–Cox transformation was used to normalize the data.

Results and discussion

In studying the green mass, a greater number of *Fusarium* CFU were discovered in the second collection at both monitored locations, as shown in Table 1.

Table 1. Presence of *Fusarium* fungi (CFU/g dry matter) in green mass, May and August, sampled at Závišice and Lukov

May		August	
Závišice	Lukov	Závišice	Lukov
31	48	470	1328

The difference between the individual collections in May and in August was statistically significant ($P = 0.04$), as can be seen in Table 2.

Table 2. Statistical evaluation of *Fusarium* fungi occurrence in green mass (ANOVA)

Effect	SS	Degrees of freedom	MS	F	*P*
Intercept	65.42674	1	65.42674	4151,306	0.009880
Locality	0.19226	1	0.19226	12,199	0.177523
Term of sampling	5.54513	1	5.54513	224,937	0.042384
Error	0.01576	1	0.01576		

There were fewer *Fusarium* spp. present in Závišice, although the difference was not statistically significant ($P = 0.18$). This difference was clearly due to the composition of the PG. While grasses constitute only 23% of the PG in Závišice, they are the dominant component (60%) of the grassland in Lukov. From the preliminary results, it is clear that the green mass contains the species *F. acuminatum, F. crookwellense, F. oxysporum, F. poae, F. sporotrichioides* and *F. subglutinans*. It is known from the professional literature that fungi of the genus *Fusarium* occur abundantly in Poaceae plants and that, in some cases, they may be active even in wintering grasslands. Inch and Gilbert (2003) recorded during their work a high percentage of *Fusarium* fungi in grasslands (up to 62%), with the most frequently occurring species being *F. graminearum*, *F. oxysporum* and *F. sporotrichioides*.

In the study of the airborne mycoflora, the statistics show that the quantity of captured *Fusarium* CFU was independent of the location and time of collection. While at both monitored locations more *Fusarium* CFU / m^2 of area were found at the August collection than at the May collection (Table 3), the differences were not statistically significant.

Table 3. Presence of *Fusarium* fungi (CFU / m²) in air, May and August, sampled at Závišice and Lukov

May		August	
Závišice	Lukov	Závišice	Lukov
2516	2359	2988	3145

The results of the analyses of the green mass and of the air are in correspondence with one another: in both cases the presence of *Fusarium* spp. was higher in August than in May. It is known from the literature that the highest concentrations of micromycete spores in air are found in summer and autumn. This fact remains true even during the kind of weather characterized by above-average precipitation that occurred during August at both locations. Preliminary species determination of *Fusarium* spp. confirmed the presence of *F. avenaceum, F. culmorum, F. oxysporum* and *F. poae* in the air. Ctvetnić and Pepeljnjak (1997) had undertaken a study of toxigenic species in the atmosphere and found, in addition to *Fusarium* spp., species from the genera *Alternaria*, *Cladosporium* and *Penicillium*. Similar airborne mycoflora, including *Fusarium* spp., were also found above mulched grasslands by Hortová *et al.* (2013). Members of the genera of microscopic fungi in the green mass and air of PG were quite similar at both monitored locations. In addition to *Fusarium* spp., the relative abundance of the class Zygomycetes; the genera *Alternaria*, *Cladosporium* and *Penicillium*; and, rather sporadically, the genera *Epicoccum* and *Stemphylium* were found. All the isolated genera of fungi are clinically significant species (Hoog *et al.*, 2000).

Acknowledgements

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Theme 3 'Novel uses of grassland, including bioenergy and biorefining'

Theme 3 invited papers

Novel products from grassland (bioenergy & biorefinery)

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Abstract

Permanent grassland can be classified into three types with regard to management intensity and productivity. High-yielding, intensively-managed, agriculturally-improved grasslands (type 1) provide biomass with qualities suitable for anaerobic fermentation and biorefinery. Its use in biogas plants is a well-established practice. Green biorefinery offers several options for biobased products, but is still in a pilot stage. Grassland biomass from semi-natural grasslands (type 2) or from landscape conservation areas (type 3) have higher lignin contents and requires pre-treatment before the fermentation or hydrolysis process can break down the cellulosic fibre. This strongly lignified biomass is also suitable for combustion or pyrolysis. However, for these conversion technologies the main problems are often unfavourably high mineral and ash contents. These novel pathways for grassland biomass can help to preserve the multi-functionality of grassland in the landscape, even without traditional livestock farming. However, the costs of harvesting, transporting, conservation and conversion of grassland biomass, especially from low-yielding areas, can be too high for a cost recovery without subsidies.

Keywords: biomass quality, biogas, combustion, pyrolysis, enzymatic hydrolysis, biorefinery

Introduction

At present, most grassland biomass is used for dairy farming. However, during the last three decades there have been notable changes in grassland use. Enhanced animal performance with increased milk and meat production have changed ruminant diet composition. On the one hand, the demand for roughage has fallen due to a higher percentage of concentrates in feed and, on the other hand, the yield of grassland is rising due to intensification. At the same time, national demand for ruminant products has decreased. In reality, there is already a surplus of grassland in many developed countries.

For a long time the only possibility to use grassland biomass for human demands was the conversion by animals to meat, milk and wool. Nowadays there are new options to use grassland biomass for energy and as biobased products. Therefore the use of grassland is becoming partly independent from livestock production. Energy generated from biomass plays a key role in the European Union's current strategies to deal with climate change and to meet the increasing energy demands (Ericsson and Nilsson, 2006). Biomass is the most multifunctional form of renewable energy, partly due to the wide range of materials which are classified as such (Roddy, 2012).

Estimates indicate a surplus grassland area of 9.2-14.9 x 10^6 ha in the European Union for the year 2020 (Prochnow *et al.*, 2009b). According to various studies (Rösch *et al.*, 2007; Hartmann *et al.*, 2011) there is a grassland surplus of about 20% in Bavaria and Baden-Württemberg. In the EU the area of surplus grassland represents about 13-22% of permanent grassland. Thus grassland could provide a proportion of 16-19% of the energy crop potential and 6-7% of the total bioenergy potential without encroaching on land needed for animal feed (Prochnow *et al.*, 2009b). Therefore, surplus grassland holds a remarkable bioenergy potential and biomass supply for energy production is regarded as one suitable way to make use of it.

In the context of a rapidly developing bioeconomy there is an increasing demand for sustainably produced biomass not only for the renewable energy sector but also for the production of

biobased products. On account of its high biodiversity, its function in carbon and nitrogen storage and its ability to fulfill several ecosystem functions (Werling *et al.*, 2014) and at the same time produce biomass for different utilization options (Rose, 2012), grassland has the potential to become an important resource for sustainable biomass supply.

In this paper an overview is given of grassland types, categorized according to their potential for biomass production, and the alternative uses are investigated.

Characterization of grassland types for alternative uses

The characteristics and yield of grassland depend on site conditions and management intensity (Dierschke *et al.*, 2002). During the last 50 years, most grassland areas with extensive grazing and mowing managements were converted into productive agriculturally-improved grasslands with high mowing frequencies (three to six cuttings) and stocking rates (Rose, 2012). The natural production of plant biomass reflects the broad range of geological, pedological and in particular the hydrological conditions in different grassland types (Dierschke *et al.*, 2002). The enhanced understanding of soil and plant nutrition, plant physiology and cultivar improvement has led to a considerable increase in grassland productivity (Hopkins and Wilkins, 2006). Agricultural intensification has also led to higher fertilizer application, in particular nitrogen and phosphorus (Rose, 2012). High-production swards can have an annual yield of 10-–12 t DM ha^{-1} y^{-1} for grazed swards and 15-20 t DM ha^{-1}y^{-1} for cut swards under good conditions. Low-production swards only yield 2-3 t DM ha^{-1} y^{-1} (Peeters, 2009).

According to the definition of Huyghe *et al.* (2014) permanent grasslands in Europe can be classified into three main types: 1) agriculturally-improved permanent grasslands, 2) semi-natural grasslands and 3) permanent grasslands no longer used for production (Figure 1). Agriculturally-improved grasslands on favourable site conditions are frequently defoliated and regularly fertilized. The management is optimized for high yields and high biomass quality. Semi-natural grasslands are lower-yielding permanent grassland areas dominated by naturally occurring grass communities. Site conditions are usually not sufficient for a more intensive grassland management. Grasslands no longer used for production are marginal areas unsuitable for agricultural production. They generally have a very low yield potential and are managed for conservation purposes.

Grassland management not only determines the yield but also the quality of the harvested biomass (see Figure1). Different pathways for novel uses of grassland biomass have different requirements on biomass quality. Demands on biomass quality may change in future on account of the continued development of conversion technologies.

The most relevant parameters are, among others, protein, lignin and ash content of the biomass. Generally, the nitrogen content increases with higher N fertilizer doses and early cutting. The lignin content is lower in intensively managed grassland (Gützloe *et al.*, 2014). In addition to management intensity, the botanical composition of grassland has a relevant impact on biomass quality. Concentrations of N, S, K, Ca, Mg are lower in grasses than in forbs (Tonn *et al.*, 2010).
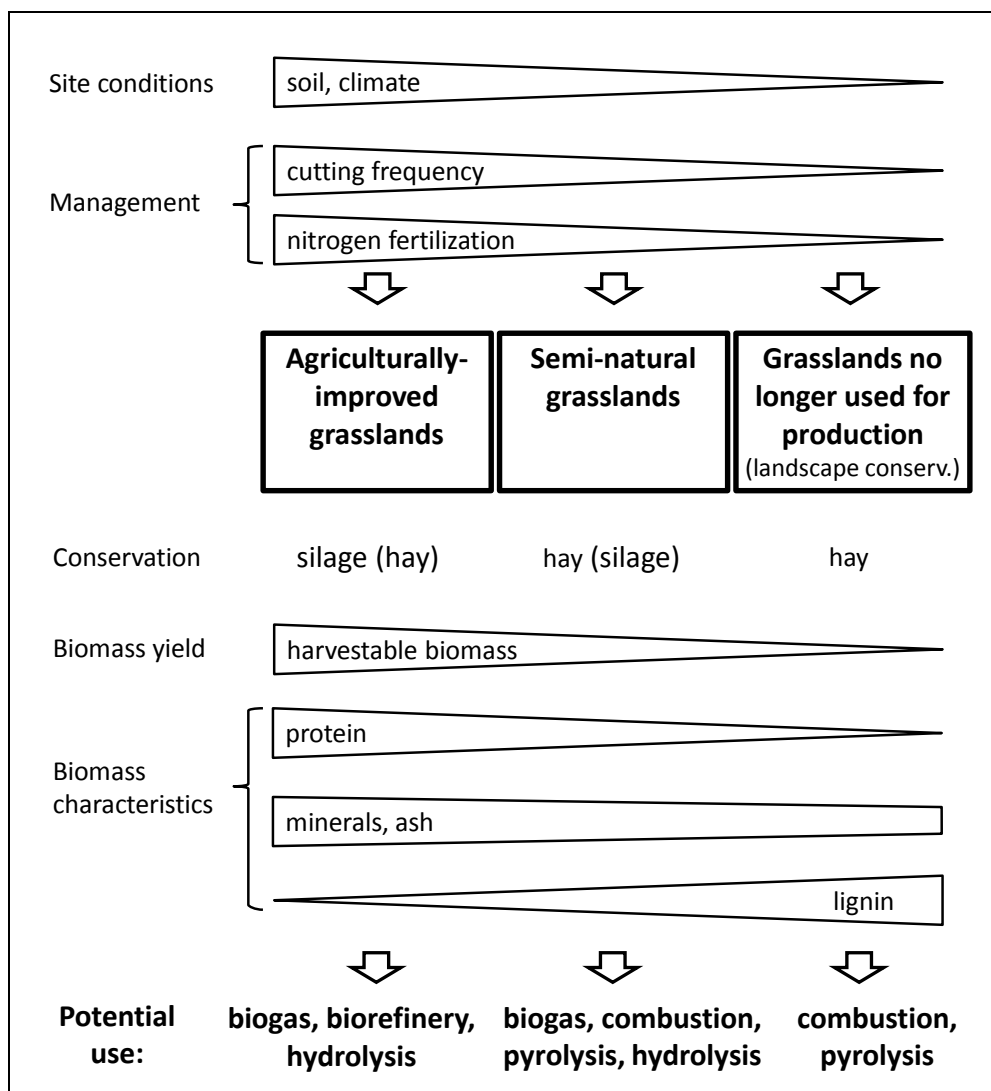
Figure 1. Characterization of permanent grassland types and pathways for biomass use

Alternative uses for biomass from permanent grassland

Figure 1 provides an overview of potential uses of grassland biomass including energetic and material applications. Grassland biomass can be processed to biofuels or bioenergy via biogas production, combustion, pyrolysis and gasification or enzymatic hydrolysis and subsequent fermentation to ethanol. For material uses or combined energetic and material uses, grassland biomass is processed through biorefinery. These options are described in the following sections.

Biogas

Today the conversion of grassland biomass by anaerobic fermentation to methane is a well-established technology. In the last decade political frameworks such as the German 'Renewable Energy Law' have supported the economic and technical progress. Maize is the most common biogas crop on account of its high yields and easy cultivation, storage and processing. In Germany the area used for maize increased by 53% in the last ten years, of which one-third is used for biogas production (FNR, 2013). The consequences are high prices of forage and rising costs for land rental in areas with biogas plants (Habermann and Breustedt, 2011).
Prochnow *et al.* (2009b) described the potential of biomass from agriculturally-improved grasslands for methane production. Effective biomass conversion depends on the botanical composition of grassland, cutting period and feedstock-specific methane yields. Shorter cutting periods increase the specific methane yields of grassland biomass. The methane yield per area

is mainly determined by biomass yield. The influence of grass species on methane yields seems to be secondary. However high nitrogen content in grassland biomass can lead to difficulties in the control and stability of the anaerobic fermentation due to high ammonium concentrations in the reactor (Andrade and Weber, 2013). Therefore a high percentage of legumes in grassland biomass and protein-rich biomass from early cuts are inappropriate for fermentation. The timing of the first cut is relevant for the biogas yield. It should not be before the vegetation stage 'ear emergence'. A first cut taken too early reduces the methane yield per hectare (Amon *et al.*, 2007). Consequently the intensity of grassland management can be lower when producing feedstock for biogas plants than in dairy production. A lower cutting frequency also reduces biomass production costs (Messner *et al.*, 2011). Energy and CO_2 balances for power generation in a CHP plant using grassland biomass showed the highest net energy yield and CO_2-equivalent reduction with two cuts per year (Gützloe *et al.*, 2014).

Low-quality biomass from semi-natural grassland or grass from landscape management can be reasonably used for fermentation if the first cut is up to late summer. Herrmann *et al.* (2013) found that methane yields from semi-natural grassland biomass decreased with later harvest from 309 l_N kg^{-1} organic dry matter in May to 60 l_N kg^{-1} in February of the following year. The economic feasibility of biogas production from landscape management grass is only given with low supply and investment costs, suitable concepts for biogas production and use, additional income from the sale of heat, and subsidies for land use (Blokhina *et al.*, 2011).

In recent years mechanical, thermal, chemical, and enzymatic pre-treatment techniques have been tested to improve the fermentation of material with higher lignocellulose content, such as straw or late-cut grassland biomass (Weiland, 2010; Michalska *et al.*, 2012). The use of pre-treated hay from semi-natural grasslands instead of silage as feedstock in biogas plants can offer new options for the storage and utilisation of grassland biomass. For example, Bauer *et al.* (2014) found a slight increase in the methane yield of hay after steam explosion treatment. The future development of these methods and subsequent assessment of their economic suitability and sustainability will reveal the opportunities that these upcoming techniques offer for the use of lignocellulosic grassland biomass for fermentation.

Another approach is the IFBB technique (integrated generation of biogas and solid fuel from biomass) for the energetic use of ensilaged biomass from semi-natural grassland (Wachendorf *et al.*, 2009; Hensgen *et al.*, 2014). After a hydrothermal conditioning process, the silage is mechanically dehydrated. The press cake, the solid fibrous fraction, can be used for combustion and the press fluid, the fraction containing easily fermentable compounds, for biogas production. Biomass conversion by IFBB has been shown to be more economically profitable than mulching as a landscape management system for the preservation of biodiversity (Blumenstein *et al.*, 2012).

Fermentation of biomass produces digestates as residues. The fertilizer value of the digestate is directly linked to the feedstock used, since there are no nutrient losses during fermentation to biogas. Degradation of organic compounds during the fermentation process leads to an increased ammonium content and therefore a higher proportion of plant-available nitrogen in the digestates (Möller and Müller, 2012). Slurry from livestock farming is frequently used for co-digestion. Slurry has a notable energy potential and helps to stabilize the fermentation process in the biogas plant (Wall *et al.*, 2013).

Combustion

Grassland biomass as a solid fuel for combustion is less favourable than biomass from perennial energy grasses and wood. It has higher contents of ash, nitrogen, sulphur, potassium and chlorine. These can lead to problems in the combustion process, such as corrosion or slagging, and to environmentally critical emissions, such as NOx, SO_2, HCl or dioxin (Lewandowski and Kicherer, 1997). Therefore late-harvested, highly-lignified and low-ash biomass is more

suitable for combustion (Prochnow *et al.*, 2009a; Iqbal and Lewandowski, 2014). Hay from late-harvested semi-natural grasslands and grasslands no longer used for production (landscape management) is more suitable for combustion than hay from agriculturally-improved grasslands due to its higher contents of lignin and lower contents of ash, potassium and chlorine. Thus extensive grassland management systems with one late cut and low fertilization are preferable when using grass as a solid biofuel. A life-cycle analysis showed that the combustion of biomass from semi-natural grassland was carbon negative and delivered a net energy gain even with low biomass yields (Tonn *et al.*, 2009). However the critical factor is the nitrogen content as this is responsible for NO_x emissions and N losses from the ecosystem. Unlike other plant nutrients, for N no recycling is possible to the soil by ash (Tonn *et al.*, 2010). Hay is very voluminous and requires compaction before it is worth transporting. From this point of view small local heating plants are more suitable than transportation of the biomass to use in a larger plant. On the other hand, co-firing in power plants with the latest filter technology can be a good pathway to use low-quality biomass. A further option is the pelleting of hay to provide a marketable product (Pilz *et al.*, 2013; Cherney and Verma, 2013). Mixed biomass pellets can help to solve grass-specific combustion problems (Nunes *et al.*, 2014).

Pyrolysis (gasification)

Thermochemical conversion by pyrolysis converts up to 75% of the energy content of plant material to a bio-oil or bio-crude (Carpenter *et al.*, 2014). These products can be further refined in a subsequent step into various base chemicals or a synthetic transportation fuel (Fischer-Tropsch liquids). There are several innovative ways of synthesis gas production and utilization which are being developed and tested in various pilot plants (Rauch *et al.*, 2014). In the bioliq® gasification process the lignocellulosic biomass is liquefied in regional plants to produce an energy-dense bio-slurry for transportation to a central gasification plant (Dahmen *et al.*, 2012). Separating the process in two steps can open up possibilities for the economic processing of plant material such as grassland biomass which is not worth being transported over long distances. Relevant feedstock quality parameters are calorific value, moisture content, proportion of fixed carbon, ash content and the minerals nitrogen, sulfur and chlorine (Robbins *et al.*, 2012). Therefore the biomass commonly used in pilot plants is wood, on account of its low ash content. Gasification technologies for low quality biomass with higher ash values are not yet well established and require further adaption (Dahmen *et al.*, 2012). The quality demands on the feedstock are similar to those for combustion. Hence biomass with high lignin content from semi-natural grasslands and grasslands no longer used for production is most suitable for pyrolysis.

Enzymatic hydrolysis

After pre-treatment of the biomass (physical, chemical) to make the lignocellulosic material accessible for the hydrolysis process, the cellulose chains are broken down by cellulase enzymes into fermentable sugars (Brodeur *et al.*, 2011). As such, the glucose yield is directly dependant on the cellulose content of the biomass (Tutt and Olt, 2011). The sugars can then be fermented to bioethanol in a subsequent step. There are several approaches for the adaption and optimization of these processes to reduce the energy demand and enzyme costs (Martín and Grossmann, 2012). Methods for the assessment of the suitability of lignocellulosic biomass are based on fermentation tests (Anderson *et al.*, 2010). For grassland biomass there are no data available for specific quality demands. Until now more homogeneous feedstocks than grassland biomass are usually used due to their easier process optimization.

Biorefinery

The aim of biorefinery is the integrated production of chemicals, fibre, food and feed products together with energy and biofuel. Green biorefinery technologies are used to exploit fresh grass

or silage-based feedstock systems. The first pilot plants started around 15 years ago in Switzerland (Grass, 2004). There are currently several green biorefinery activities being pursued in the Netherlands, Denmark, Austria and Germany (Mandl, 2010). An overview of the potential use of grassland biomass in biorefineries was given by O'Keeffe (2010) for Ireland. To date green biorefinery projects are mostly in the pilot phase and not yet economically sustainable. However the increase in activities in the last decade points to the opportunities and potentials of this pathway (Cherubini, 2010).

Biorefinery can be defined as 'the sustainable processing of biomass into a spectrum of marketable products and energy" (IEA Bioenergy, 2009). In green biorefinery the primary step is the mechanical fractionation of the biomass by pressing. The green juice (fresh material) or brown juice (silage) extracted and press cake recovered are used for the further processing of various products and energy (Figure 2). Decomposition methods (enzymatic, fermentative, hydrolytic, thermal, chemical) are sometimes applied before fractionation. The freshly-pressed green juice contains several components including proteins, lipids, glycoproteins, lectins, sugars, amino acids, dyes, minerals and enzymes. In addition silage juice contains relatively high concentrations of lactic acid, which can be used for the production of plastics, and inorganic salts (Kamm *et al.*, 2010). The press cake is a fibrous fraction, which can be used as raw material for products such as insulation material, peat substitution products in horticulture, bio-composites, pulp and paper, and thermoplastics.
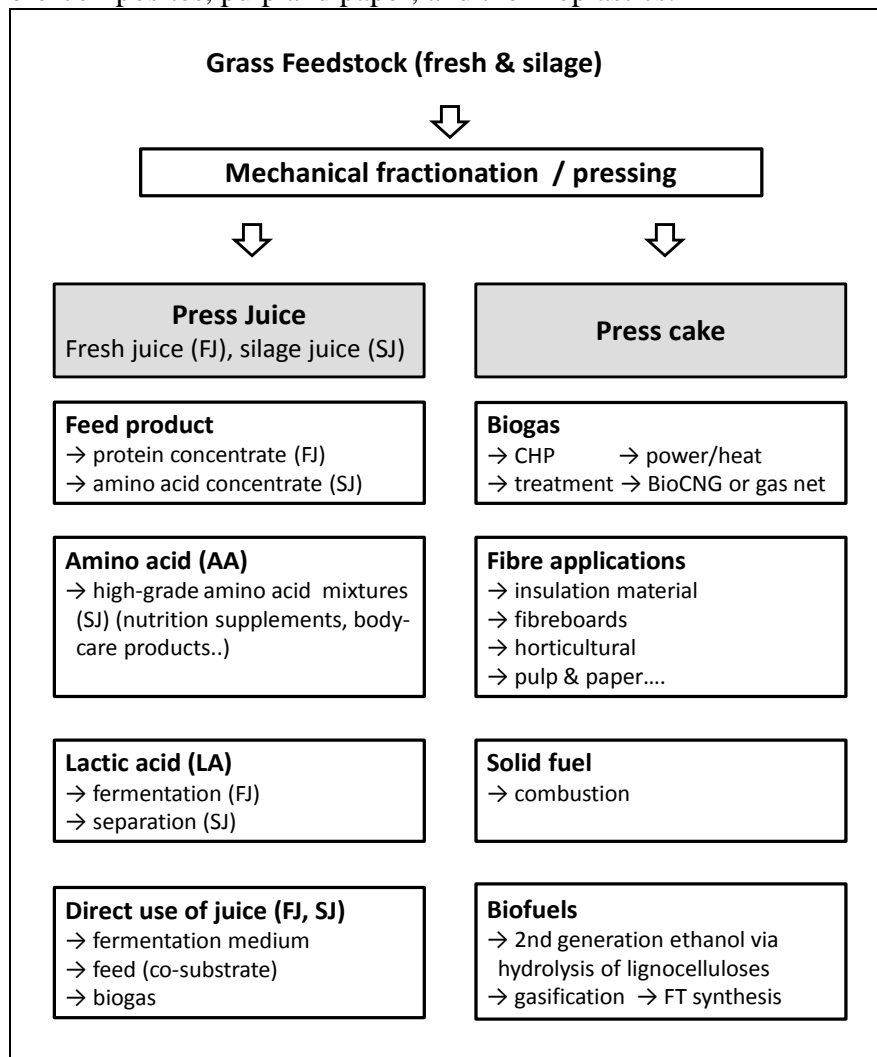
Figure 2. Possible biorefinery products from different feedstock fractions (Mandl, 2010)

One main challenge is the generation of pure chemical products such as ethanol, organic acids or amino acids by different separation technologies (Kamm *et al.*, 2010). Residues remaining after refining processes can be used as feedstock for energy production (biogas, combustion) or as fertilizer. The quality demands on the raw material depend on the intended product. For example, in a biorefinery model producing protein feed for animals and insulation material, the most important quality parameters for grass feedstock are fibre and protein content (Grass, 2004). Biomass from semi-natural grasslands normally has a too low content of exploitable ingredients and is not suitable for conservation as silage. The low content of exploitable components in fresh biomass from semi-natural grasslands normally renders it unsuitable for biorefinery. Therefore it is to be expected that only biomass from agriculturally-improved grasslands can be used in biorefinery processes. The achievement of dedicated quality demands can require special management or botanical composition of the meadows.

The economic situation of green biorefinery systems has been evaluated in some studies (O'Keeffe, 2010; Höltinger *et al.*, 2014). The variable operating costs, which include the costs of feedstock fractioning and downstream processes, are most important for the economy of green biorefineries. One question which remains to be answered is how to optimize the input volume of biorefinery facilities. High capacity plants have lower processing costs per biomass unit, but high costs for feedstock logistics. Also the energy balance between consumption and production will be a determining factor in the success of a biorefinery (O'Keeffe *et al.*, 2012). For sustainable and truly green final products the use of sustainable biomass is not enough; protection of the environment requires methods and techniques with minimized impact on the environment (Cherubini, 2010).

Conclusions

Permanent grassland can supply biomass for energetic and material uses. However, grassland is also the main source of feed for ruminants. Therefore the availability and cost of grassland biomass for novel uses depend on the economic benefits of grass-based livestock systems and landscape and agrarian structure. For grassland areas with conditions which make harvesting of biomass by machinery problematic, traditional pasture systems are more suitable. Different grassland types provide biomass of varying quality. Biomass from agriculturally-improved grasslands is easily degradable and appropriate for fermentation in biogas plants or conversion to biobased products in biorefineries. Fermentation of grassland biomass is widespread in several European countries. New challenges are the adaption of the process for biomass with higher lignin contents from semi-natural grasslands. It is possible to use this biomass and also that from landscape conservation for combustion in adapted heating plants. New options for producing second-generation biofuel by pyrolysis and enzymatic hydrolysis are still in a developmental stage. There are promising perspectives for converting low-quality grassland biomass in advanced facilities in future. Further challenges are the integration of these new value chains in the landscape without the loss of other functions of grassland.

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Grasslands for forage and bioenergy use: traits and biotechnological implications

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Abstract

Growing energy crops on marginal lands will avoid land use needed for food crops and use this unused marginal land which is unfavourable for food production. Perennial rhizomatous grasses (PerRhG) have the advantage that several of the species of interest for biomass and bioenergy production can be grown on marginal land types. However, it will be necessary to improve management practices and to select for plant genotypes which are able to tolerate the stressful conditions they are exposed to. This review looks at the current state of genomics and biotechnology improvements of PerRhG species and the morphological and physiological traits of perennial rhizomatous grasses that could potentially be modified to maximize biomass production on marginal land. The traits include aspects of crop phenology, canopy and leaf photosynthesis, biomass partitioning, nutrient and water use efficiency, cold and salt tolerance, and moisture and chemical content. It is proposed that newly developed biotechnological methods combined with high-throughput plant phenotyping offer opportunities to rapidly select new genotypes that could achieve economic yields on large areas of marginal land.

Keywords: biomass production, perennial grasses, plant traits, environmental stress, biotechnology

Introduction

The development of forage and bioenergy grass species which have a suite of desirable physical and chemical traits while maximizing biomass yields is challenging. Achieving this will depend on identifying the fundamental constraints on productivity and addressing these constraints using modern genomic breeding tools to identify and exploit suitable crops and to optimize the management of these crops to maximize biomass production. Biomass production, water and nitrogen use, tolerance to biotic and abiotic stress and low soil fertility are critical factors in selection of ideal bioenergy feed stocks (Bressan *et al.*, 2011; Xin and Wang, 2011). Ideally, these perennial grasses should be also doing well on marginal lands that are unfavourable for food production and that are currently unused or underutilized. The aim therefore is to identify and breed the most highly productive plant species that can be grown on the various types of marginal land and to optimize production practices. Marginal land is land that is of poor quality for agriculture and which yields poor economic returns for the farmer. The cultivation of grass on degraded or exhausted agricultural soils can serve to restore soil organic carbon and improve its physical properties (Potter *et al.*, 1999). Crops growing on marginal land are subjected to a range of abiotic stresses, including shortage/excess of soil water, poor nutrient availability, salinity and high and low temperatures, which reduce their yield to below their potential. Because these stresses vary from area to area a diverse range of feedstocks suitable for different niches are necessary and feed into breeding objectives (Yuan *et al.*, 2011).

In the case of several field crops, such as maize and oilseed rape, they have undergone much genetic improvement, and significant increases in yields have been achieved in the last fifty years. This selective breeding has gone hand in hand with improved management practices and

in many cases the relative contributions of management and breeding have been about equal (Richards, 2000; 2004). However, in the case of several forages and second generation biofuel crops such as perennial rhizomatous grasses (PerRhGs) most attention until now has been on improved management. The candidate PerRhGs are either largely undomesticated and have not undergone the selective breeding that characterises our current major food crops, or they have been selected to maximize quality and productivity for grazing animals (Heaton *et al*., 2008; Vermerris, 2008).

The first, and arguably the most important, management choice affecting biomass production on marginal land is the choice of species or variety to be used for biomass production.

Irrespective of the physical stress which renders land marginal, biomass production can be maximized by utilizing the genetic variability that exists among and within species and choosing the species/genotype likely to give the highest yield under a specific physical stress.

Conventional biotechnology to improve traits

General principles

In most breeding schemes (with exceptions, e.g. mutation breeding) at least one round of recombination is required to introduce novel genetic variation by rearrangement of alleles by meiotic recombination. This recombination step with succeeding evaluation and selection defines one cycle in breeding. One breeding cycle can take between two to six years to complete. For most grass breeding schemes formal grass breeding began only ~ 1900 in Europe and USA, and about 20 to 25 breeding cycles have been completed in 100 years. Compared to maize breeding, which often uses two to three seasons per year, in winter nurseries over 100 breeding cycles have been completed since modern breeding started and has led, in a selection experiment, to a 4-fold increase in oil content (Dudley 2007). Thus there is a huge untapped potential for breeding of forage and bioenergy grasses. This untapped potential could be much more utilised in conjunction with Marker Assisted Breeding (MAS) Strategies to breed by phenotypic selection and with molecular marker support at the same time. The objective of molecular markers in breeding programmes should be to describe the effect of the marker on phenotypic trait performance. Unfortunately the situation for many agriculturally important traits is far from trivial since many of these traits are controlled by quantitative trait loci (QTL) which can affect up to a few hundred genes.

A strategic decision needs to be taken if selection is carried out with a few markers, which has been termed marker assisted selection, or if many markers at a time, which are spread out throughout the entire genome, are being selected, which is termed genomic selection (GS). Genomic selection follows a marker index score which sums up all individual marker effects to arrive at a total score. A prerequisite to implement any type of marker assisted selection is that advances in genomics of the species under question have been made. These advances include the generation of collections of different types of molecular markers, expressed sequences tags (ESTs), annotated transcriptomes, complete genomes or shut gun sequences of genomes. GS requires a substantial amount of markers fairly evenly spread and at high density across the genome.

Examples of state of art in genomics for one perennial grass species: Miscanthus

Some recent research has used gene expression analysis to understand phenotypic variation in *Miscanthus* using methods such as RNA-Seq. Chouvarine *et al.* (2012) used transcriptome sequencing of rhizome samples to generate an exome sequence database for *Miscanthus* complete with gene ontology functional annotations. Barling *et al.* (2013) also generated a comprehensive expressed sequence tag (EST) catalogue using RNA-Seq that was predicted to represent a high proportion of the *Miscanthus* transcriptome using comparisons to sorghum

gene models. They also analysed expression profiles in rhizomes characterized in the spring compared to the autumn to reveal biological pathways that exhibit altered regulation. Some candidate gene work has also been undertaken to understand variation in important lignin-related genes (Suman *et al.*, 2011). Other research has focused on generating genetic linkage maps of *Miscanthus* that are needed for several applications such as QTL analysis and MAS. High-resolution maps based on sequence markers allow the use of QTL accessible from other grass species through alignment based on syntenic relationships (Ma *et al.*, 2012). However, such maps have only recently been produced. Higher resolution genetic maps of *Miscanthus* species based on DNA sequence markers have recently been generated using next-generation sequencing technology (Ma *et al.*, 2012; Swaninathan *et al.*, 2012). This has allowed for data transferability and several comparative genomic analyses. Association mapping (linkage disequilibrium mapping) is a method of mapping QTLs that takes advantage of historic linkage disequilibrium to link phenotypes to genotypes (Myles *et al.*, 2009). The genome is sampled for markers (such as SNPs) and associations statistically detected between markers and a particular phenotype. Associations are independently verified to show that they 1) directly contribute to the trait of interest, or 2) are linked to a QTL that contributes to the trait of interest. Association mapping in the form of a genome-wide association study (GWAS) is an advance on standard association mapping and has been most widely applied to the study of human disease, cattle breeding and more recently to plants including *Miscanthus* (Slavov *et al.*, 2013).

Genetic modification (GM) to improve traits

General principle

GM crops are obtained by genetic engineering techniques. Genetic engineering offers an opportunity to generate unique or novel genetic variations that could not otherwise be obtained by conventional breeding (Wang and Ge, 2006). Furthermore, transgenesis has become an indispensible tool for understanding basic biological questions (Dixon *et al.*, 2007). The generation of transgenic plants, coupled with selection, has led to the development of transgenic cultivars in several major cash crops, such as maize, soybean and cotton. These transgenic cultivars have been widely adopted in many parts of the world. Transgenic technology has also been used to improve forage and turf species (Wang and Ge, 2006) and, more recently, to improve biofuel production from potential bioenergy grasses.

Genetic transformation methods for perennial rhizomatous grasses

Although the first proven transgenic forage grass was obtained by direct gene transfer to protoplasts (Wang *et al.*, 1992), this method is rarely used nowadays because protoplasts are difficult to handle and regenerate. The two main methods currently used to produce transgenic plants are: microprojectile bombardment (biolistics) and *Agrobacterium*-mediated transformation.

Biolistics utilizes high-velocity gold or tungsten particles to deliver exogenous DNA into the plant cells for stable transformation. *Agrobacterium tumefaciens* is a soil bacterium that harbors a tumor-inducing (Ti) plasmid. For the purposes of genetic transformation, the T-DNA of the Ti plasmid is replaced with foreign genes intended for incorporation into the plant cells. Compared to biolistic transformation, *Agrobacterium*-mediated transformation allows stable integration of the transgene into the plant genome in a relatively lower copy number and generally leads to fewer rearrangements and more stable transgene expression over generations (Dai *et al.*, 2001; Hu *et al.*, 2003). *Agrobacterium*-mediated transformation was originally used for dicotyledonous plants. Starting from the late 1990s, *Agrobacteria* have been successfully used to transform grasses. To date, transgenic plants have been obtained for many forage, turf and bioenergy species, such as tall fescue, meadow fescue, red fescue, perennial ryegrass,

Italian ryegrass, creeping bentgrass, Kentucky bluegrass, orchardgrass, bahiagrass, zoysiagrass, switchgrass (Wang and Ge, 2006; Wang and Brummer, 2012).

Example of genetic improvement of perennial grasses by transgenic technology

Cell wall composition directly affects processing properties of lignocellulosic biomass. Due to the association of lignin with cellulose and hemicellulose, cell wall materials are largely recalcitrant to hydrolytic enzymes. To solve the cell wall recalcitrance problem, a direct and effective approach is to modify lignin content/composition by down-regulation of the enzymes involved in lignin biosynthesis (Hisano *et al.*, 2009). In switchgrass, the down-regulation caffeic acid methyltransferase (COMT) led to reduced lignin content and altered lignin composition, improved forage quality, and up to a 38% increase in ethanol yield using conventional biomass fermentation processes (Fu *et al.*, 2011). Furthermore, the transgenic lines required less severe pretreatment and less cellulase for equivalent ethanol yield compared to unmodified switchgrass (Fu *et al.*, 2011). The transgenic COMT plants were transferred to the field. Under field conditions, transgenic switchgrass were phenotypically normal and continued to show reduced lignin content, increased sugar release and improved ethanol yield (Baxter *et al.*, 2014).

Biomass yield is an important but complex trait for both forage and bioenergy purposes. The identification and manipulation of major regulatory genes that govern the expression of a group of downstream genes provide an effective way to improve complex traits (Fu *et al.*, 2012). In recent years, miRNAs have emerged as a prominent class of gene regulatory factors (Zhang *et al.*, 2006). Plant miR156 is a family of small, non-coding, endogenous RNAs with a relatively high expression level in the juvenile phase of plants (Matts *et al.*, 2010). A miR156b precursor was overexpressed in switchgrass (Fu *et al.*, 2012). The degree of morphological alterations of the transgenic switchgrass depends on miR156 level. Relatively low levels of miR156 overexpression were sufficient to increase biomass yield while producing plants with normal flowering time. Moderate levels of miR156 led to improved biomass but the plants were non-flowering. These two groups of plants produced 58-101% more biomass yield compared with the nontransgenic control (Fu *et al.*, 2012). The improvement in biomass yield was mainly because of the increase in tiller number (Fu *et al.*, 2012). The non-flowering phenotype offers an effective approach for transgene containment in grasses.

Regulatory problems/concerns

A major limitation for deployment of transgenics is the complicated GMO regulatory processes (Wang and Brummer, 2012). Despite the wide adoption and the beneficial economic and environmental impacts of major transgenic crops (e.g. corn, soybean, cotton, canola), it has been extremely difficult to deregulate and commercialize forage, turf and bioenergy crops. These crops do not enter the food chain directly; their potential environmental or ecological impacts are the main focus of risk assessment. Most widely grown forage, turf and bioenergy species are highly self-incompatible and outcrossing. Pollen-mediated transgene flow is a major concern for such outcrossing species. The impacts of regulations on research and environmental studies of transgenic forage, turf and bioenergy crops have been summarized by Strauss *et al.* (2010) and Wang and Brummer (2012). Several biological containment measures have been developed or proposed to control transgene flow. Such measures include male sterility, seed sterility, maternal inheritance, delayed flowering or non-flowering (Wang and Brummer, 2012). In addition, the cisgenic or intragenic approach may provide a cost-effective way for genetic engineering and commercialization of grasses (Wang and Brummer, 2012).

Traits to be improved and targets for improvement

Physiological and morphological traits

Plants have evolved functional traits or features which reflect their ecological strategies in a particular environment. A plant functional trait is therefore any morphological, physiological or phenological feature, measureable in the plant at the cell to the whole organism level, which potentially affects its fitness (Perez-Harguindeguy *et al.*, 2013). Phenology (the timing of developmental stages in the plant life cycle), leaf area dynamics, radiation interception and utilization, and crop growth and partitioning are major processes that determine the harvestable biomass of energy crops. These processes are regulated by morphological and physiological traits and maximising yield is about optimising these processes. Yield potential for crops grown on marginal land is reduced below the optimal by stresses and the aim here is to identify plant traits which either allow avoidance or increase tolerance of these stresses. Breeding new varieties which possess these traits depends on identifying measurable physiological traits which can be used as selection criteria. The aim is to select appropriate species and genotypes which are adapted to local soil and climatic conditions. Selection criteria need to be based on the triple goals of maximizing productivity, minimizing inputs and maximizing utilization for energy production. Some of the traits of particular interest in recently instigated breeding programmes are drought tolerance (Clifton-Brown *et al.*, 2002), frost tolerance (Clifton-Brown and Lewandowski, 2000), maintenance of growth at low temperature (Farrell *et al*., 2006), chemical composition (Lewandowski *et al.*, 2003), resistance to pests and diseases (Clifton-Brown *et al*., 2008), altering plant architectural features such as dwarf structure and erect leaves (Zhu *et al*., 2010) and differences in photosynthetic capacity (Carver and Hocking, 2001).

After Karp and Shield (2008) are three main challenges in achieving yield improvement. (1) There should be a reduction in the thermal threshold for growth of the canopy leaves which extends the growing season. Second, above-ground biomass should be increased without depleting the below-ground biomass so much that there are insufficient reserves available for the next years' growth. Third, above-ground biomass should be increased without restricting growth due to excess water depletion and developing water stress. Traditional plant breeding, selection and hybridization techniques are slow and for some PerRhGs there is a limited availability of germplasm. *Miscanthus x giganteus*, for example, is a sterile triploid, which is normally propagated from rhizome pieces. There has, however, been some long-term conventional breeding of another C_4 grass, switchgrass (Casler, 2012), which has produced large yield gains. In the future, new biotechnological routes may produce even greater improvements. Genetically modified (GM) energy crop species may be more acceptable to the public than are GM food crops, particularly in Europe (Koh and Ghazoul, 2008), but there are still concerns about the environmental impact of such plants including gene flow from non-native to native plant relatives. Consequently non-GM biotechnologies may be more attractive. Initially it is likely that the use of molecular biology will focus on the use of molecular markers that can be used in the rapid screening of germplasm within the breeding population. However, linking these molecular markers to complex traits such as yield is difficult because yield is controlled by many genes. With the advent of cost-effective and rapid sequencing technologies, there is currently an expansion of knowledge of genes and their expression profiles in potential biofuel crops including PerRhGs (Ma *et al*., 2012; Swaminathan *et al.*, 2012). In addition, there has been recent development of automated greenhouse systems for high-throughput plant phenotyping which allows the non-destructive screening of plants over a period of time by means of image acquisition techniques. This involves applying sophisticated image analysis algorithms (Hartmann *et al.*, 2011) to extract several phenotypic parameters related to growth, yield and stress tolerance and as a consequence reduces the tedious and time consuming manual analysis of large numbers of phenotypes. Marker-assisted selection (MAS) is the process of

using DNA, biochemical or morphological markers as indirect selection criteria for selecting agriculturally important traits in crop breeding. The use of MAS for manipulating simple/qualitative traits is relatively straight forward but its use for the improvement of complex/polygenic traits, including plant tolerance/resistance to abiotic stresses is far more complicated. Quantitative trait loci (QTL) mapping and MAS methods will utilize high-density single nucleotide polymorphism (SNP) data including genic SNPs. A recent development for MAS is genome-wide selection that uses high-density genotypic SNP data to find haplotype blocks associated with traits. This approach has been used successfully in cattle and should offer high potential in forage and bioenergy grasses (Nakaya and Isobe, 2012).

The utilization of these techniques will allow us to identify in PerRhGs the physiological and morphological traits that are most important in determining yield and stress tolerance of grasses grown on marginal land and to suggest how they might be used as selection criteria in screening and breeding programmes. Biomass production and partitioning of biomass to different parts of the plant can be viewed as three steps. In the first step the processes involved are canopy development and light interception. In the second step intercepted light energy is converted into biomass, and in the third step biomass is partitioned into different part of the plant (Zhu *et al.*, 2010). The yield that a crop can achieve under optimal management practices and in the absence of biotic and abiotic stresses is dependent on the amount of incident radiation, the efficiency of interception of that radiation by the canopy, and the efficiency of photosynthetic conversion of light energy into dry matter (Jones, 2011). In the following sections we review the key morphological and physiological processes which determine these traits.

Phenological stages of development

Phenology describes the plant life cycle and it is linked to environmental drivers, particularly accumulated temperature measured as cumulative growing degree days (GDD) (Larcher, 2003). This measure of thermal period is computed as an average of daily maximum and minimum temperatures above the base temperature for growth of a particular species. The GDD are determined by seasonal and interannual variations in weather and the phenological stages are assessed in terms of GDD (Larcher, 2003). Grasses progress through a series of phenological stages during the year and to maximize production it may be necessary to select for optimal phasing of these. For example, extending the reproductive phase may result in greater stem extension, and therefore increased standing biomass. Extending crop duration is the simplest genetic way to increase total photosynthesis during the growing season leading to higher crop biomass and yield. This is because longer crop duration increases the solar radiation interception during the crop growth period. However, not only can the duration of growth be manipulated to increase biomass and yield, but also its timing. Providing there are no other limiting factors, full light interception should be achieved by the time that daily solar radiation has reached its maximum. Therefore the need is to manipulate phenology to better match periods of highest solar radiation. Timing may also be important to increase yield and biomass in relation to water supply. Therefore, if water is a major limiting factor it may be beneficial to maximise growth when temperatures are lower and vapour pressure deficit is low as this is when transpiration is lowest and water use efficiency is highest (Richards, 2004).

Flowering time has a key role in determining the quantity and quality of the harvested biomass and considerable variation in flowering time among genotypes of some species has been demonstrated (Jensen *et al.*, 2011). Early flowering shortens the effective length of the growing season, thus reducing biomass production but where flowering does not occur before the autumn frosts, the onset of senescence and remobilisation of nutrients appears less efficient in some species such as *Miscanthus* (Clifton-Brown *et al.*, 2008). This can result in higher ash contents on combustion which are naturally associated with higher uptakes of elements such as

N, P and K. Late flowering has also been associated with higher over-winter losses of *Miscanthus* genotypes in the first year following planting (Clifton-Brown *et al.,* 2008).

Canopy and leaf photosynthesis

Leaf area development and canopy light interception is dependent on the number of leaves that develop per unit ground area and the rate at which leaves expand. The appearance of leaves and their rate of expansion are driven largely by temperature. Significant variation in the temperature threshold for leaf appearance and subsequent leaf expansion has been demonstrated in *Miscanthus* genotypes (Clifton-Brown and Jones, 1997; Farrell *et al.*, 2006). The effect of expansion at lower temperatures is to allow earlier development of the canopy in spring and increase the length of the growing season. This increases the total amount of radiation intercepted by the canopy during the growing season and has the potential to increase yield significantly if leaf photosynthetic activity is maintained. In a study comparing the canopy duration among a diverse collection of 244 *Miscanthus* genotypes, Robson *et al*. (2013) found that yield was positively correlated with canopy duration as were both early establishment and later senescence.

The process of photosynthesis is pivotal to the production of biomass and there is considerable variation in photosynthetic rates among crops and genotypes within them, particularly at low temperature (Naidu and Long, 2004). For example, *Miscanthus* can maintain 80% higher photosynthetic light-use efficiency than maize when grown at 14/11°C (day/night) (Naidu *et al*., 2003). The photosynthetic conversion efficiency of light energy is the combined gross photosynthesis of all the leaves within the canopy, less all the plant respiratory losses. It might be assumed therefore that there is a correlation between maximum leaf photosynthesis and yield. Unfortunately, most research has failed to clearly demonstrate this correlation although it has been shown recently that increasing photosynthesis using elevated CO_2 concentration does indeed result in higher yields under field conditions (Long *et al.*, 2006). Under the right circumstances it would appear therefore that there is a yield advantage associated with selecting for higher leaf photosynthetic rates. However, the measurement of photosynthesis is plagued by problems of integration over the life cycle of the plant (Horton, 2000). Spot measurements vary with leaf age, leaf position, time of day, light intensity, plant health and development stage. It is therefore important to obtain an integrated assessment of photosynthesis of the whole plant rather than of single leaves.

Biomass partitioning and assimilate distribution

In PerRhGs used as energy crops virtually all of the above-ground material is harvested. However, the below-ground roots and rhizomes are important stores of carbon which enable the spring growth of shoots, and nutrients which are recycled throughout the growing season into the shoots. The dynamics of carbon movement between shoots and rhizomes and roots show a seasonal pattern (Heaton *et al.*, 2009). Consequently, when photosynthate is in short supply, for example during rapid stem extension in the spring, the reserves of carbon in the rhizomes may be important to support continued growth.

PerRhGs develop extensive root systems which, in effect, transfer atmospheric carbon into soil biomass on their senescence and death. In perennial systems where the soil is not ploughed this results in substantial sequestration of the carbon in the soil. A number of studies have shown that annual rates of soil carbon sequestration range between 1-10 t/ha/yr (Zimmerman *et al.*, 2012). Variation in sequestration appears to be influenced by the depth of the roots as well as the soil characteristics.

Nutrient use efficiency

In PerRhGs, nutrient use efficiency (NUE) is strongly influenced by the annual recycling of nutrients from the above-ground biomass to the rhizome system. The nutrient that most

frequently limits growth of energy crops is nitrogen. NUE is normally expressed as the ratio of increase in plant biomass to increase in plant nitrogen over the growing season. In energy crops this approximates to the ratio of biomass to nitrogen content at the end of the growing season (Xu *et al.*, 2012). Nitrogen use efficiency can be improved at three levels in perennial species. First, NUE is enhanced by increasing the amount of biomass produced per unit of nitrogen invested into the photosynthetic apparatus, which is different in C_3 and C_4 plants (Jones, 2011). Second, NUE can be enhanced by increasing the fraction of soil nutrients that are taken up by the plant (Lewandowski and Schmidt, 2006). Third, NUE is enhanced by increasing the fraction of nitrogen translocated out of the leaf canopy and stems during senescence; translocated nitrogen can then be stored in the rhizomes for use in the following year (Beale and Long, 1997). This recycling process has the effect of reducing, and in some cases eliminating the need to use nitrogenous fertilizers (Christian *et al.*, 2008). The recycling occurs late in the growing season, during the senescence of the crop and is most effective when the crop is harvested in the late autumn or winter. Consequently, late senescence or stay-green characteristics of PerRhGs are clearly linked with higher nutrient use efficiencies. Limiting steps in plant nitrogen metabolism are different under high and low N levels. At high N inputs major variation in NUE is due to differences in N uptake but at low N variations in NUE are dependent on changes in N utilization and in the remobilization of N. For PerRhGs cultivated on marginal land it is therefore more likely that the most important traits to focus on will be those associated with high N utilization and remobilization capacity.

Water use efficiency

The water use efficiency (WUE) of a biomass crop is the yield of harvested product achieved from the water made available to the crop through precipitation and/or irrigation (Richards, 2004). It is known for several crop species that genotypes with higher WUE have higher concentrations of the stable isotope $\delta^{13}C$ (Richards, 2004). This is because the enzymatic processes of photosynthesis discriminate against ^{13}C in the atmosphere and in genotypes with higher WUE this discrimination is diminished since a higher proportion of CO_2 in cells is utilized and consequently more $^{13}CO_2$ is fixed (Condon *et al.*, 2004). Plants are analysed for variability in the relative ratio of $^{13}C/^{12}C$ expressed as $\delta^{13}C$ relative to a standard and the stable isotope ratios can be used for screening cultivars/genotypes for higher WUE.

Drought and flooding tolerance

Globally, drought-induced losses in crop yield exceed losses from all other causes (Jones, 1988, Richards, 2004). Drought tolerance is the ability of plant tissues to maintain metabolism at low water potentials brought about by water stress. Drought tolerance can be achieved by careful selection of genotypes, breeding new varieties using methods such as MAS and using management practices such as the exogenous application of hormones or osmoprotectants (Farooq *et al.*, 2009). Tolerance is often associated with the accumulation of solutes within cells which has the effect of retaining cell turgor (Jones, 1988). Flooding tolerance is little studied although flooding is a widely distributed problem and may become increasingly important under climate change with the increase in frequency of extreme events. Flooded environments present challenges for biomass production as crop growth can be reduced in wet conditions when oxygen concentrations in the root zone are reduced. However, some species such as *Phalaris arundinacea* are well adapted to waterlogged conditions and Etherington (1984) found differences in flooding tolerance among genotypes of *Dactylis glomerata*. More recently, some PerRhGs have been shown to have the potential capacity to mitigate flooding. Macleod *et al.* (2013) showed that novel *Festulolium* cultivars (*Lolium perenne* x *Festuca pratensis*) can significantly reduce run-off during flooding compared to commercially used *Lolium* and

Festuca cultivars and this was shown to be due to intense initial root growth followed by rapid senescence at depth.

Cold tolerance

There are vast areas of marginal land in the cool-temperate climatic zone, particularly in Eurasia and North America, which could potentially be cultivated to meet a future demand for productive bioenergy crops. Biomass production from grasses cultivated in environments with low temperatures may be reduced by a combination of a shorter growing season and poor growth at low temperatures. Gudliefsson *et al*. (1986) found significant differences in cold hardiness and ice tolerance when ten pasture grasses were tested in controlled environments. Timothy (*Phleum pratense*) and Kentucky bluegrass (*Poa pratensis*) proved to be the most resilient to these stresses while orchardgrass (*Dactylis glomerata*) and reed canary grass (*Phalaris arundinacea*) proved least resilient to these stresses. Indigenous varieties of *Lolium perenne* were found to be more winter hardy compared to more exotic varieties even if they came from colder climates (Lorenzetti *et al.*, 1971). Clifton-Brown and Lewandowski (2000) demonstrated cold tolerance variation between species and genotypes of *Miscanthus*. Thus, selection of genotypes with enhanced low temperature tolerance represents an effective strategy for maximising biomass production in environments affected by cold stress.

Salt tolerance

Salt stress is today in Europe mainly a problem in southern and south-eastern regions such as Hungary, Romania and Spain (Tóth *et al.*, 2008), but the areas may increase with climate change. Extensive genetic diversity for salt tolerance exists in plant taxa from halophytes which are native flora of saline environments to glycophytes which are salt sensitive or hypersensitive. Most crops are glycophytes (Yokoi *et al.*, 2002). However, there is considerable variation in salt tolerance both within and between species of glycophytes, including grasses. If rankings of salt tolerance in different publications are compared, the order of the species varies, as most rankings are based on one or a few varieties per species. Great variation, not only between species but also between varieties within each species, has been observed (Grare, 2010). Different management practices can be used to alleviate the effects of salt; however, the most practical and, arguably, the most cost-effective means of managing salt stress is the choice of salt tolerant genotypes.

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Theme 3 submitted papers

Bioenergy potential of meadows of Ukraine

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Abstract

We present results of a study of the bioenergy potential of the Ukrainian meadows, an area of about 6.6 million ha plus 10 million ha of fallow lands. The average energy production of unimproved meadows is 2.2 Mg ha^{-1} of dry biomass or 30.6 J ha^{-1} of heating (gross) energy. Economically viable annual energy potential of these meadows is 4.22 million Mg or 12% of total biomass potential. Meadow degradation is caused by the overgrowth of thick-stemmed herbs, shrubs and low forest. The ways of improving their energy production are shown further. After including legumes into grass mixtures or adding N_{120} to sown grass stands, the energy production of sown meadows increased to 5.93-12.07 Mg ha^{-1} of dry biomass or to 107.4-213,1 J ha^{-1} of heating energy. That is 1.3-1.9 times more than from sown grass stand, and 2.4-3.5 times more compared to natural grass stand without improvement of $P_{60}K_{90}$ under the same conditions. The sown legume-grass is the most productive stand, involving different varieties of *Trifolium pratense* L., and in the case of liming, *Medicago sativa* L.

Keywords: bioenergy, biomass, meadows, potential, productivity, sown grass stand.

Introduction

In the process of energy dependence reduction of Ukraine, the development and use of renewable energy resources for biofuels, including plant biomass, is of great importance. Its part in total production of primary energy in Ukraine remains low, at only 1.24%. However, Ukraine has a large economically feasible annual potential of biomass available for energy use, estimated at 34 million Mg of reference fuel, which is 18% of total energy consumption (Geletukha *et al.*, 2013). The Energy Strategy of Ukraine for the Period to 2030 (2006) suggests increasing the part of biomass in total energy consumption up to 10%. Economically viable annual energy potential of perennial herbaceous plant communities is estimated at 7 million Mg of reference fuel, which is 20% of total biomass potential, including 4.22 million Mg (or 12%) from meadows (Kumrgak *et al.*, 2013). Therefore, in the context of stable development, the usage of grassland biomass for energy, which in Ukraine is about 6.6 million ha, demands deserves special attention. In view of the catastrophic reduction in livestock for fodder production, their biomass is hardly ever used. However, until recent time, current studies on energy potential of Ukrainian meadows and development of measures to improve their energy production have not been carried out.

Materials and methods

We have conducted a study on the energy potential of meadows according to generally accepted field and laboratory methods, using geobotanical, measuring and weighing, calculating-comparative, chemical and mathematical-statistical methods. Content of heating or gross energy was calculated according to the chemical composition of dry biomass. In terms of heating energy, 1 Mg of reference fuel was compared to 1 Mg of coal. To assess the impact of species composition and variety assortment of legume-grass mixtures in energy production of sown grass stand, we conducted our research during 2011-2013, against the background of annual addition of $P_{60}K_{90}$. We conducted research on dry, wet, and drained meadows (village of Lytvynivka, Vyshgorodskyi district, Kyiv region). The soil type we studied is soddy gley, sandy loam, in 0-15 cm layer; pH 4.8; K is 7.3 mg and P is 3.1 mg per 100 g of soil. In the

research we used new recognized varieties of perennial legumes and grasses of selection of the NSC 'Institute of Agriculture NAAS'. Weather conditions during the years of study were very contrasting; summer days were dry and hot. The most favorable atmospheric moisture for grassland cenosis was in the year of 2012.

Results and discussion

The geo-botanical surveys generally showed that unimproved meadows with natural grass swards in their present condition are characterized by low energy production. This is due to the lack of effective measures for their improvement and use. The average energy efficiency of unimproved meadows is 2.2 Mg ha^{-1} of dry biomass or 30.6 J ha^{-1} of heating (gross) energy, including Polissia (2.7 Mg ha^{-1}) of dry mass, forest-steppe (3.8) and steppe (1.7 Mg ha^{-1}) (Petrychenko and Kurhak, 2013). The most productive are lowland meadows, and the least productive are upland meadows. Annually the total area of unimproved meadows in Ukraine accumulates approximately 14 million Mg of dry biomass, or 240 million J of heating energy or 7 million Mg of reference fuel, and with fallow lands 22 million Mg and 370 million J and 11 million Mg. respectively. Addition of mineral fertilizers can increase their productivity and increase heating energy output by 2-3 times.

In contrast to the 1990s, the degradation of meadows in Ukraine is due to the lack of cattle that would otherwise feed on the grasslands. Meadows are overgrown with thick-stemmed herbs. Those which border on the forest are overgrown with low forest and bushes; after 5-6 years they are of little use for mowing for fodder, but their attraction for energy remains.

Among trees and shrubs the most widespread are *Salix triandra* L., *Alnus glutinosa* (L.) Gaertn and *Betula pendula* Roth. Among grasslands are thick-stemmed plants (genera: *Cirsium, Rumex, Stenactis, Solidago, Urtica*) and in the south *Ambrosia, etc.*) which can successfully be used for energy purposes.

Our studies on the drained flood meadows of Polissia have shown that the creation of sown meadow grass swards with the use of symbiotic nitrogen from perennial legumes, or from nitrogen fertilizers, increases their energy efficiency by several times. A study was conducted of the influence of species and varietal composition of grass components on the productivity of a sown clover-grass mixture. Analysis of the results showed that the output of 1 hectare of dry biomass on average for 2011-2013, against the backdrop of annual addition of P$_{60}$K$_{90}$ to these sown legume-grass swards, ranged from 5.93 to 11.22 Mg, heating energy was from 107.4 to 199.1 J. The most productive appeared to be the sown grass stand involving *Dactylis glomerata* L. (cv. Kyiv Early and Natalka), *Festuca pratensis* Huds, (Rosynka and Siveryanka) and *Bromopsis inermis* (Leys.) Holub (Arsen and Helius) Adding N$_{120}$ to the control sown grass swards involving *Festuca pratensis* (cv. Siveryanka) and *Lolium perenne* L. (cv. Leta) increased meadow productivity from 4.68 to 10.41 Mg ha^{-1} of dry mass or by 2.2 times. Natural grass swards without improvement on the same background P$_{60}$K$_{90}$ were by 1.7-3.4 times less productive compared to sown grass and clover-grass swards.

The second research on the same flood drained meadows when adding P$_{60}$K$_{90}$ included a study on energy production of sown swards with the inclusion of legume-grass mixtures or adding different species and new varieties of perennial legumes to grass plants (Table 1). On these swards, on average for 2012-2013, the addition of P$_{60}$K$_{90}$ obtained 8.32-12.07 Mg per ha of dry biomass, or 145.8-213.1 J of heating energy, which is 1.3-1.9 times greater than from sown grass swards, and by 2.4-3.5 times greater compared to natural grass swards without improvement. The most efficient were sown legume-grass swards with *Trifolium pratense*, cv. Polyanka or cv. Polisyanka and, on the limed soil, *Medicago sativa* cv. Olga or cv. Intensive 174. Liming of soil increased productivity of swards by 0.08-2.57 Mg ha^{-1} of dry mass. The best response to liming was shown by grass mixtures containing *Medicago sativa*, and the poorest response was from mixtures with *Lotus ukrainicus* Klok.

Table 1. Energy production of sown legume-grass mixtures in 2012-2013.

Kinds and varieties of grass mixtures standard quantity of seed rates, kg ha^{-1}	Without liming			Limed background		
	Dry mass, Mg ha^{-1}	Heating energy, J h^{-1}	Reference fuel, Mg ha^{-1}	Dry mass, Mg ha^{-1}	Heating energy, J ha^{-1}	Reference fuel, Mg ha^{-1}
Trifolium pratense Polyanka, 9 + grass plants*	10.63	186.3	5.32	11.78	200.3	5.89
Trifolium pratense Polisyanka, 9 + grass plants*	10.60	177.0	5.30	11.65	206.2	5.83
Trifolium pratense Polyanka, 4.5 + Polisyanka, 4.5 + grass plants*	11.32	199.6	5.66	12.07	213.1	6.04
Medicago sativa Olga, 10 + grass plants*	8.36	147.7	4.18	10.97	192.5	5.49
Medicago sativa Intensive 174, 10 + grass plants *	8.44	150.5	4.22	11.01	195.4	5.51
Medicago sativa Olga, 5 and Intensive 174, 5 + grass plants *	8.72	155.5	4.36	11.14	197.1	5.57
Lotus ukrainicus Local, 5 + grass plants *	8.60	150.1	4.30	9.20	163.3	4.60
Lotus ukrainicus Local, 2.5 + *Trifolium pratense* Polyanka, 4.5 + grass plants *	11.01	192.4	5.50	11.09	196.0	5.55
Trifolium repens L. Sprynt, 5 + grass plants *	8.32	145.8	4.16	8.11	142.7	4.06
grass plants *	6.18	109.5	3.09	6.85	120.5	3.43
grass plants * + N$_{120}$	10.97	192.2	5.49	11.59	196.6	5.80
Natural grass stand	3.41	56.5	1.71	3.57	62.1	1.79
HIP$_{0/05}$	0.72			0.82		

* plants: *Bromopsis inermis* Arsen, 10+ *Festuca pratensis* Siveryanka, 8 + *Phleum pratense* Argenta, 6 kg ha^{-1}

According to our data, the heating energy content in 1 kg of dry biomass of different sown grassland swards hardly depended on the species and varietal composition of mixtures, and was equal to 17.51-18.16 MJ, which is equivalent to the energy value of by-products (straw) of perennial grasses and winter wheat.

Conclusions

The economically feasible energy potential of meadows is 4.22 million Mg of reference fuel or 12% of total energy consumption. The average energy production of unimproved Ukrainian meadows with natural grassland swards is 2.2 Mg ha^{-1} and that of flood meadows of Polissia is 3.4 Mg·ha^{-1} of dry biomass. Addition of legumes to grass mixtures, or of N$_{120}$ to the sown grass swards, increases the energy efficiency of meadows by 1.7-3.5 times.

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Permanent grassland for anaerobic digestion: a novel insight into management–methane yield relations

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Abstract

Grassland biomass as feedstock for biogas production is increasingly considered as an alternative use for grassland. Therefore, the joint research project 'GNUT-Biogas' aims at the identification of optimal management intensities for seven productive grassland types in Germany. Emphasis was placed on the effects of management intensity on the feedstock-specific methane yield. Results show that methane yields of grassland biomass can vary widely and are considerably influenced by management options. Higher cutting frequencies and early cuts result in low lignin content and consequential high feedstock-specific methane yields. The management variant corresponding to the production of high quality fodder for dairy cattle resulted in highest biomass qualities for biogas production. Further aspects have to be considered for a final conclusion on optimal grassland management for anaerobic digestion.

Keywords: grass silage, biogas production, management intensity, digestibility, acid detergent lignin

Introduction

Grassland biomass has increasingly been considered as feedstock for biogas production in recent years. Grass silage has been shown to be a capable feedstock for anaerobic digestion (Prochnow *et al.*, 2009; McEniry and O'Kiely, 2013). However, grassland biomass can differ considerably in quality and suitability for biogas production. The most relevant parameter that characterizes quality of biomass for biogas production is the feedstock-specific methane yield. It is influenced by several factors such as vegetation type and composition, intensity of grassland management, mechanical pretreatment during harvesting, and ensiling and storage (Prochnow *et al.*, 2009). The aim of the joint research project 'GNUT-Biogas' is to identify the optimal management intensity for seven productive permanent grassland types in Germany considering biomass yield and quality. The present paper focuses on the effects of management variants on biomass quality.

Materials and methods

Biomass of seven productive grassland types was obtained from different typical sites in Germany. On each site, four management variants were compared in a randomized block experiment with four replications. Management variants differed in cutting frequency, time of cutting and level of fertilization and correspond to the production of:
(1) high quality fodder for dairy cattle (180 – 300 kg N ha^{-1}; 4 - 5 cuts per year with the first 3 cuts harvested before mid-July);
(2) high quality fodder for dairy cattle with reduced level of nitrogen fertilization (120 – 220 kg N ha^{-1}; 3 - 4 cuts per year with the first 3 cuts harvested before mid-July);
(3) biomass utilizing local resources (110 – 200 kg N ha^{-1}; 3 - 4 cuts per year with the first 2 cuts harvested during transition from vegetative to generative stage of growth); and
(4) biomass focusing on maintenance of characteristic species composition (120 – 220 kg N ha^{-1}; 3 - 4 cuts per year with the initial cut early at the onset of grazing and the second cut after flowering of dominant species).

A detailed description of grassland types, site conditions and management variants is given by Schmidt *et al.* (2012). Harvested biomasses were wilted, chopped and ensiled in 1-litre glass silos. After storage for a period of 90 days, silages were analysed for chemical composition and feedstock-specific methane yield as described in detail by Herrmann *et al.* (2011). Methane yield was analysed in triplicates per cut in batch anaerobic digestion tests under mesophilic conditions. Batch tests were conducted for at least 30 days, but were not interrupted until daily gas production was below 0.5 % of the total gas production for 3 consecutive days.

Results and discussion

Feedstock-specific methane yields of grassland biomasses varied widely from 255 to 402 l_N kg_{ODM}^{-1}. Table 1 shows feedstock-specific methane yields of three selected grassland types in two years of harvest.

Table 1. Effect of management intensity on methane yield (l_N kg_{ODM}^{-1}) of 3 grassland types in 2 years of harvest. Data show mean (standard deviation) of 3 replicates.

Management variant	Cut	*Lolio-Cynosuretum,* typical character		*Trisetetum* without *sylvaticum*	*flavescentis, Geranium*	*Alopecuretum pratensis,* periodically flooded	
		Year 2011	Year 2012	Year 2011	Year 2012	Year 2011	Year 2012
1	1st	390.7 (2.1)	367.2 (1.8)	341.4 (2.5)	349.8 (2.6)	367.2 (2.8)	308.6 (3.6)
	2nd	378.2 (1.9)	364.2 (1.2)	323.6 (7.2)	295.0 (3.3)	314.7 (1.3)	297.9 (3.5)
	3rd	357.7 (5.0)	317.4 (3.1)	329.3 (1.9)	306.5 (5.8)	282.1 (4.1)	272.5 (5.3)
	4th	332.9 (1.1)	351.1 (3.0)	304.6 (3.7)	318.1 (0.7)	279.1 (2.6)	284.5 (0.7)
	5th	361.4 (5.9)	294.0 (2.9)				
2	1st	360.7 (3.6)	356.7 (4.5)	352.9 (4.1)	348.9 (2.8)	358.1 (3.4)	302.2 (2.4)
	2nd	365.3 (4.6)	334.2 (6.6)	297.9 (7.4)	301.0 (0.4)	308.3 (2.8)	289.0 (1.3)
	3rd	343.8 (1.3)	329.2 (1.1)	323.3 (2.0)	293.1 (0.3)	282.3 (3.0)	284.6 (0.4)
	4th	310.4 (2.3)	303.0 (3.6)	317.2 (3.8)	304.9 (1.3)	278.6 (3.6)	287.5 (2.9)
3	1st	349.6 (1.1)	347.0 (5.4)	341.3 (4.3)	336.8 (0.9)	303.3 (3.9)	312.4 (0.8)
	2nd	348.5 (1.7)	309.9 (1.8)	294.3 (7.6)	303.9 (0.3)	269.8 (0.9)	278.2 (1.2)
	3rd	338.8 (2.3)	305.7 (1.2)	316.0 (3.8)	300.2 (2.7)	275.1 (2.4)	255.1 (2.9)
	4th	342.1 (3.9)	309.5 (3.3)				
4	1st	402.0 (2.0)	366.9 (2.8)	356.0 (7.7)	358.2 (3.7)	279.3 (6.3)	284.6 (1.2)
	2nd	333.3 (2.5)	297.2 (0.8)	293.1 (5.5)	274.6 (1.2)	286.1 (5.5)	293.2 (2.4)
	3rd	316.0 (2.5)	307.0 (0.9)	327.1 (0.8)	312.2 (3.2)	293.0 (7.1)	271.1 (4.0)
	4th	344.1 (4.6)	342.1 (3.2)				

Highest feedstock-specific methane yields were achieved by the *Lolio-Cynosuretum* vegetation whereas *Alopecuretum pratensis* revealed lowest overall biomass quality (Table 1). Feedstock-specific methane yield was considerably influenced by grassland management. Early cut grassland biomass, which was the first cut of management variant (1) and variant (4), with exception of the *Alopecuretum pratensis* vegetation, showed highest feedstock-specific methane production. With ongoing maturity or days of growth until harvest feedstock-specific methane yield tended to decrease. This is in accordance to findings of previous studies that observed negative impacts of advancing harvest dates on methane production of common grass species (McEniry and O'Kiely, 2013). Lower feedstock-specific methane yields with advancing stages of maturity are attributed to an increase in less digestible cell wall fractions (Prochnow *et al.*, 2009). A relation between acid detergent lignin (ADL) content, which varied between 17 and 76 g kg_{DM}^{-1}, and feedstock-specific methane yield was found ($R^2 = 0.551$; data not shown). Comparison of variants indicate that grassland management for the production of high quality fodder for dairy cattle also results in highest quality for biogas production, i.e. highest mean feedstock-specific methane yields of grassland biomasses. N fertilization only marginally affected methane production. A mean increase in feedstock-specific methane yield of 1 % was observed for management variant (1) with higher N fertilization compared with variant (2) at

similar harvest conditions. In contrast, weather conditions within the growth period may considerably affect biomass quality. Feedstock-specific methane yields were higher in year 2011 (Table 1), which was characterized by high rainfall especially during June and July, whereas the year 2012 revealed lower precipitation compared with the long term average.

Conclusion

Biomasses from permanent grassland reveal large differences in feedstock-specific methane yield depending on grassland type, management intensity and time of cutting. Methane production is mainly affected by the lignin content of the biomasses. High cutting frequencies and early cuts lead to grassland biomass with low lignin content and high feedstock-specific methane yields. Grassland management corresponding to the production of high quality fodder for dairy cattle resulted in highest biomass qualities; however, further aspects such as biomass yield, maintenance of vegetation type, energetic and economic efficiency, and ecological performance have to be considered for final conclusions on optimal grassland management.

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Potential use of native *Piptatherum miliaceum* (L.) Coss. for forage production and bioenergy

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Abstract

The use of dual-purpose plants for forage production and bioenergy in Mediterranean agricultural system may enhance their flexibility and increase the competitiveness of agro-enterprises. *Piptatherum miliaceum* (L.) Coss. is a native species of Sardinian pastures. It has good palatability for ruminants in the early stage of growth, while the senescent stems and leaves may be used for bioenergy production. A preliminary characterization of Sardinian germplasm was performed in a field experiment. Eleven natural populations were compared, using tall fescue and cocksfoot varieties as references, for aboveground biomass characteristics and phenology. The dry matter yield of *P. miliaceum* ranged from 1254 to 1900 g plant^{-1}, while the moisture content of its biomass ranged from 38 to 46%. The aboveground biomass was allocated mainly in tillers. The quality of biomass was slightly lower than that of the conventional bioenergy species *Panicum virgatum* L. *Piptatherum miliaceum* showed a growing season one month longer than tall fescue and cocksfoot. The long growing season, the high biomass production, the ability to survive to summer drought and the quality of biomass of *P. miliaceum* are promising traits for the use of this species for both forage and bioenergy production.

Keywords: native perennial grasses, smilo grass, forage production, bioenergy

Introduction

The availability of plant species that increase the flexibility of agricultural systems and offer to farmers the opportunity to differentiate their income is one of the strategies that may increase the competitiveness of agro-enterprises. *Piptatherum miliaceum* (L.) Coss. (smilo grass) is a herbaceous native perennial grass in dry Mediterranean environments. It is common in natural pastures and it can be found in marginal environments such as semiarid mining zones (Conesa *et al*., 2006). It has good palatability for ruminants in an early stage of growth, but becomes unpalatable in the old stands (Baldoni *et al*., 1974). Its high biomass production offers the chance to use the stems and leaves for bioenergy production. To our knowledge, no bio-agronomic characterization for bioenergy production of smilo grass has been performed to date. The main objective of this study was to perform a preliminary characterization of some Sardinian smilo grass accessions for phenology, biomass production and quality.

Materials and methods

The experiment was carried out in Sassari (40° 45' 15" N 8° 25' 13" E), altitude 24 m a.s.l., on an alluvial soil with pH 7.8. Average total annual rainfall for the site is 550 mm, mainly concentrated in autumn and spring. Seeds of smilo grass collected from wild populations growing in Sardinia (Italy) during summer 2011 were grown in pots from the end of summer up to April 2012, when seedlings were transplanted into the field. Plots (2 m × 1 m) of 11 accessions of smilo grass were established in a completely randomized design with three replicates. Plant distance was 0.5 × 0.5 m and 8 plants per accession were transplanted in each plot. *Festuca arundinacea* Screb. (tall fescue) cv. Fletcha and *Dactylis glomerata* L. (cocksfoot) cv. Jana were used as control species. No irrigation or herbicides were applied after transplanting.

Phenology of plants was observed weekly to define the growing stages in the experimental field conditions. All plots were harvested in 2012 and 2013, once a year in July, when smilo grass leaves dried out. The measurements of biomass and its components were made on two plants per plot. With the aim of estimating biomass partitioning, the dry weights of leaves, tillers and spikes were measured after drying the separated samples in an oven at 60 °C to constant weight and water content. The analysis of biomass quality was done determining hemicellulose, cellulose, lignin and ash contents according to Van Soest *et al.* (1991). Data were analysed by ANOVA using Statgraphics Centurion software. Data were transformed when needed and homoscedasticity of data was tested by Bartlett's test. Sample means were compared by Tukey's HSD test.

Results and discussion

In the first year, plant yields were negligible, both in smilo grass and in conventional grass species. In the second year, tall fescue and smilo grass showed higher plant dry weights than cocksfoot (Table 1).

Table 1. Dry matter yield (g plant $^{-1}$), moisture content at harvest (%), biomass partitioning (% DW) and full flowering date of 11 smilo grass accessions (PM), 1 cocksfoot (DA) and tall fescue (FE) varieties measured in 2nd year. Biomass was cut on July 2013. Different letters indicate statistical differences at $P \leq 0.05$ (Tukey test).

Species	Dry matter yield (g plant^{-1})	Moisture content at harvest (%)	Tiller mass ratio (% DW)	Leaf mass ratio (% DW)	Spike mass ratio (% DW)	Full flowering date in 2013
PM13SEM	1360.3 ab	44.5 ab	58.2 a	22.6 bc	19.3 a	14 Jun
PM14PZS	1592.1 ab	38.4 bc	59.1 a	19.5 c	21.4 a	14 Jun
PM15VNM	1676.4 ab	39.4 bc	62.6 a	20.5 c	16.9 a	28 Jun
PM16SLR	1855.9 a	44.1 abc	61.2 a	20.3 c	18.5 a	7 Jun
PM18FRT	1732.9 ab	39.8 abc	58.6 a	24.3 bc	17.1 a	7 Jun
PM19CMC	1435.1 ab	44.7 ab	59.5 a	17.0 c	23.5 a	7 Jun
PM20PMT	1323.0 ab	40.2 abc	56.2 a	16.6 c	27.2 a	7 Jun
PM21DCM	1916.1 a	45.1 ab	56.3 a	19.3 c	24.4 a	7 Jun
PM22STG	1254.7 ab	45.9 a	55.4 a	19.0 c	25.6 a	7 Jun
PM23ZCN	1691.7 ab	45.1 ab	61.0 a	20.7 bc	18.3 a	7 Jun
PM24MNR	1648.7 ab	43.1 abc	57.5 a	20.0 c	22.5 a	7 Jun
DA29JA	263.3 c	36.8 c	44.2 b	37.7 a	18.1 a	3 may
FE28FL	825.9 bc	41.3 abc	64.2 a	31.2 ab	4.6 b	3 may

However, smilo grass yields ranged from 1254 to 1900 g plant^{-1} while tall fescue yield was about 826 g plant^{-1}. The aboveground biomass was allocated mainly in tillers, both in conventional species and smilo grass, with *F. arundinacea* showing the highest tiller mass ratio (64%), followed by smilo grass (59%) and finally, by cocksfoot (44%); that was the only species with statistically significant different values in tiller mass ratio. The biomass partitioning resulted favourable for bioenergy uses, where a higher ratio of stems is required. In smilo grass, the relatively high moisture content at harvest was due to the tillers, as they also remained green during summer in drought conditions. After the first cut in July 2012, smilo grass showed a fast regrowth and in September some plants flowered again but did not produce seeds. Vegetative growth extended from the end of August 2012 to the end of April 2013 for the early-flowering accessions and up to the third decade of May for the late-flowering accession PM15VNM. Heading was a long phase, lasting for one month before the full-flowering stage. Full flowering occurred in the first decade of June in most accessions, whereas two of them flowered in the second decade and only one in the last decade of June-beginning of July. Smilo grass accessions

showed a growing season extended a month more than cocksfoot and tall fescue. These latter, in fact, flowered in the first decade of May.

Regarding the biomass quality of smilo grass components (Table 2), all calculated parameters were consistent with those reported for the conventional bioenergy species *Panicum virgatum* L. (switchgrass) by McKendry (2002). The ash content of tillers, spikes and leaves was higher than reported for switchgrass.

Table 2. Hemicellulose, cellulose, lignin and ash contents in leaves, stems and spikes of a smilo grass accession.

	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Ash (%)
Leaves	23.59	40.67	3.41	16.23
Stems	26.58	39.15	9.44	5.34
Spikes	36.32	28.01	9.93	5.24

Conclusions

The long growing season, the high biomass production of smilo grass, its ability to survive in summer drought conditions and the quality of biomass for bioenergy production are promising traits for the use of this species for both forage and bioenergy production. A follow-up of this research is needed to exploit the variability of agronomical and phenological traits found among and within accessions and to establish the better crop management to combine forage and bioenergy production.

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Second generation bioethanol production from *Phalaris aquatica* L. energy crop

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Abstract

Nowadays the inevitable deficit of fossil fuels, the increasing energy consumption and the security of global energy market are the driving forces to utilize renewable energy resources, such as biomass, for bioenergy, biofuels and bio products. One type is lignocellulosic biomass that can be found in abundance in perennial forage species. The purpose of this study was to investigate aboveground biomass production and lignocellulosic concentration stability of rainfed crop (5-years old) from native Mediterranean perennial grass *Phalaris aquatica* L. at a lowland region of Central Greece as potential dedicated energy crop, and to investigate the integrated biochemical conversion processes efficiency of lignocellulosic biomass into second generation bioethanol. The results indicated that *Phalaris aquatica* L. energy crop could serve as a stable second generation bioethanol feedstock at lowland areas of Central Greece after the establishment year due to high biomass yield and cell wall concentration. The results also revealed that under the integrated biochemical conversion pathway (dilute acid pre-treatment, enzymatic hydrolysis, fermentation) glucose monomer derived from cell wall carbohydrates could efficiently (> 80%) be converted to bioethanol.

Keywords: perennial grasses, cellulosic biomass, pretreatment, bioconversion, bioethanol.

Introduction

The production of bioethanol has attracted worldwide attention as a strategy for combating global warming and improving European energy security. This has placed attention on the utilization of fermentable sugars derived from lignocellulose, the largest known renewable carbohydrate source for biofuel production (Jorgensen *et al.*, 2007). Herbaceous species such as perennial grasses are promising lignocellulosic feedstocks due to their high yield potential, low establishment cost and higher carbohydrate content than annual species. However, plant cell wall recalcitrance necessitates the efficient treatment in order to release the fermentable sugar monomers. Dilute acid pretreatment followed by enzymatic hydrolysis is the typical process used to convert lignocellulosic biomass to fermentable sugars (Iranmahboob *et al.*, 2002). The objectives of the present study were: a) to evaluate aboveground biomass yield and cell wall concentration stability of *Phalaris aquatica* L. energy crop for five successive years (2008 – 2012) in Central Greece, and b) to investigate the bioconversion efficiency into bioethanol through the integrated biochemical processes.

Materials and methods

Biomass samples of perennial grass (*Phalaris aquatica* L. - Harding Grass, HG) rainfed plantation (5-years old) in Central Greece, located at Pteleos site at 15 m altitude, were collected at the end of the growing season for five successive years (2008-2012). Biomass production was measured using 20 (1×1 m^2) plots. Harvested biomass was dried at 60 °C for 48h, milled to a size of 1 mm and subjected to fibre analyses 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. Air dried biomass samples at 10% (w/v) solid

loading were mixed at mild pretreatment conditions (1.5% (w/v) H_2SO_4, 120 °C, 45 min) in 50ml screw cap bottles in oil bath. Solid residues recovered by filtration were hydrolyzed with cellulases mixture (Celluclast 1.5L and Novozyme 188). Enzymatic hydrolysis was performed in 250ml flasks with 100 ml of 0.05 M sodium citrate buffer (pH = 4.8) at 50 °C in an incubator at 150 rpm agitation. Kinetic analysis was performed at different biomass loading (2, 4, 6 % w/v) with enzyme loading (30 FPU/g biomass) and ratio 1:2 for 72 h. Afterwards, the glucose found in the hydrolysate was fermented by the yeast *Saccharomyces cerevisiae* Sigma (Type II) in 250ml flasks, incubated at 30 °C, pH = 6.5 at 150 rpm for 34h. Glucose and ethanol concentration were analysed by HPLC. All experiments were performed in duplicate. One-way ANOVA was used to compare biomass production and cell wall concentration of HG at different years. Further differences were evaluated with the LSD post hoc test, at a level of significance of 0.05 (Kinnear and Gray, 2008).

Results and discussion

Aboveground biomass production (t DM ha^{-1}) of *Phalaris aquatica* L. crop varied significantly between the years 2008 - 2012 (Table 1). Dry matter yield increased from the first season after establishment to the second growing season. In the second year, yield increased by 661% (13.8 t ha^{-1}), in the third year it was further increased by 45% , and in the next two years the increase was more stable, at 5.5% and 6.2 % respectively. The average dry matter yield for the 5-year period was 15.8 t ha^{-1}. The same trend in biomass yield during several growing seasons were reported for perennial grass species such as reed canary grass (Sahramma *et al.*, 2003) and *Mischanthus x giganteus* (Clifton-Brown *et al.*, 2007). Growth year had a significant effect on cell wall compositon of HG aboveground biomass (Table 1).

Table 1. Mean biomass yield and cell wall concentration of *Phalaris aquatica L.* crop at Pteleos site for five successive years (2008-2012).

Year	Biomass yield (Mg ha^{-1} DM)	NDF (g kg^{-1} DM)	ADF (g kg^{-1} DM)	ADL (g kg^{-1} DM)	Cellulose (g kg^{-1} DM)	Hemicellulose (g kg^{-1} DM)	Total structural carbohydrates (g kg^{-1} DM)
2008	1.8c	666c	339c	78a	261b	327c	588c
2009	13.8b	795a	503a	72a	292a	431a	723a
2010	19.9a	752b	474a	64b	278a	410a	688b
2011	21.0a	765b	507a	76a	258b	431a	689b
2012	22.3a	760b	482a	73a	277	409a	687b
Mean	15.8	748	468	72.5	279	396b	675
s.e.d	3.8	21.7	31.1	2.4	6.2	19.3	22.8

a,b: Means followed by the same letter do not differ statistically significant (LSD Test, *P*<0.05)

NDF, ADF and cellulose were higher in the subsequent growth years after the establishment year, with the loss of hemicellulose during this period owing to the increased deposition of cellulose. Significant annual variation of cell wall concentration from other perennial grasses grown for biomass, have been also reported (Schmer *et al.,* 2010; Allison *et al.*, 2012).

The chemical composition of feedstock has a major influence on the efficiency of bioethanol generation. Dilute sulphuric acid pretreatment at mild conditions had significant impact on *Phalaris aquatica* cell wall, as increased cellulose by 45.5% and decreased hemicellulose by 79.4% and lignin by 13.9%, respectively (Pappas, 2010). According to Zheng *et al.*, (2009) promotion of cellulose content and demotion of hemicellulose and lignin content can facilitate the enzymatic hydrolysis in perennial grasses. The increase of biomass loading resulted in linear increase of glucose in the enzymatic hydrolysate (Figure 1), with the maximum concentration of 18.8 g/l found at 6 % biomass loading at 48h. After hydrolysate condensation, glucose concentration reached 22.6 g/l and was used as a carbon source for ethanol production by yeast

fermentation. The fermentation resulted in 84.3% yield (ethanol concentration equal to 5.49 g/l) based on the theoretical maximum yield and 0.50 g/l/h ethanol productivity.
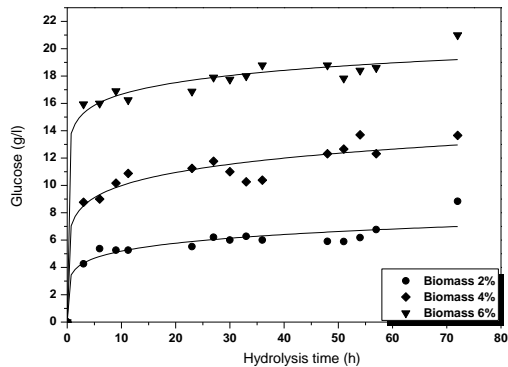
Figure 1. Kinetics of glucose concentration (g/l) at different biomass loading

Conclusion

The results indicated that *Phalaris aquatica* L. as an energy crop could serve as a second-generation bioethanol feedstock in lowland areas of Central Greece after the establishment year, due to high and stable biomass yield and cell wall concentration. Furthermore, moderate acid pretreatment followed by enzymatic hydrolysis is an effective biochemical method to release sugar monomers from *Phalaris aquatica* L. cell wall carbohydrates which can be efficiently fermented to bioethanol.

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Hydrothermal processing of rush (*Juncus* spp.) and bracken (*Pteridium aquilinum*) dominant biomass from semi-natural landscape management

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Abstract

Semi-natural habitats that are dominated by rush (*Juncus* spp.) and bracken (*Pteridium aquilinum*) show an increase in floristic diversity following management through cutting and biomass removal. In this study the waste biomass created is utilized for energy and product production. Biomass samples from rush- and bracken-dominated vegetation were converted by hydrothermal carbonization and hydrothermal liquefaction to derive oil, hydrochar, gaseous and aqueous fractions. Prior to this conversion the biomass had been ensiled, washed with water, and mechanically dehydrating using a screw press. This process produced a press cake with a reduced mineral composition compared to the silage. The process method had a significant impact on the oil, hydrochar and gas yields; liquefaction provided a higher oil composition, and carbonization a higher hydrochar composition. Washing and pressing did not have a significant impact upon the product yields following conversion via carbonization or liquefaction, but did impact on mineral composition. The community type (rush or bracken) had a significant impact upon the oil yield with the rush-dominant biomass providing a higher oil yield.

Keywords: hydrothermal, *Juncus*, bracken, hydrochar, oil

Introduction

The indigenous vegetation communities found in the UK uplands support ecosystems with international conservation significance. Cutting management may be implemented in order to preserve biodiversity on these areas with the resultant accumulation of waste biomass. This biomass could potentially be available for further processing for energy or product development (Corton *et al.*, 2013). Two common habitats that benefit from a cutting and biomass-removal management system are rush- (*Juncus* spp) and bracken- (*Pteridium aquilinum*) dominated semi-natural landscapes. Bracken- and rush-dominant biomass has been investigated as a resource for energy and product conversion as part of the EU PROGRASS project (Wachendorf *et al.*, 2009). The associated process involved ensiling the biomass, washing the feedstock with water and mechanically dehydrating it to produce a press cake. This procedure reduced the mineral composition of the biomass in order to improve its combustion fuel qualities.
Hydrothermal conversion is a method of processing biomass in order to obtain products for energy generation, carbon sequestration or chemical production. Superheated water is created by heating water under pressure within a vessel; during hydrothermal conversion the vessel also contains biomass. Under these conditions the biomass is converted into a multiple product mix that consists of aqueous, oil, gaseous and hydrochar fractions. By conducting hydrothermal conversion at different temperatures, oils (favoured in hydrothermal liquefaction) or hydrochars (favoured in hydrothermal carbonization) can be increased at the expense of other fractions in the product mix. In this study, biomass from rush- and bracken-dominated habitats, in the form of silage and press cake, were subjected to hydrothermal carbonization and liquefaction. This paper summarizes the impact of hydrothermal conversion route (carbonization or liquefaction),

pre-treatment methods (silage or press cake) and community type (rush or bracken-dominant) upon the product yields.

Materials and methods

The biomass tested had been cut from replicated plots of each vegetation type located at the Pwllpeiran Upland Research Centre, Wales, and had been ensiled without pre-treatment in experimental silos. The experimental work was conducted at the laboratories of the Energy Research Institute at the University of Leeds. Conversion via hydrothermal carbonization and hydrothermal liquefaction was performed in a 75 ml capacity, unstirred, bomb-type batch reactor (Parr, USA). The temperature and pressure inside the reactor was monitored using a Parr 4836 controller. The reactor was hydrocharged with ~3 g of sample and 27 ml of distilled water; this was pre-mixed and added to the reactor as slurry prior to sealing and heating. To achieve hydrothermal carbonization the residence time was set at one hour and the reactor temperature was set at 250 °C with a pressure of 44 bar. To achieve hydrothermal liquefaction the reactor temperature was set at 350 °C with a pressure of 170 bar and the residence time was one hour. Separation using dichloromethane, water and filtering were employed. The residence time was measured from the point at which the reactor reaches 250 °C or 350 °C respectively. The heating rate of the reactor was approximately 10 °C per minute and the reactor was purged with nitrogen prior to processing. Separation procedures are detailed in Figure 1. GenStat® Release 13 was employed for discerning the levels of significance using an analysis of variance.

Results and discussion

The oil yield was lower when biomass derived from the bracken plots was employed compared to that from the rush plots, but the hydrochar yields were the same (Table 1).

Table 1. The impact of vegetation community, hydrothermal conversion route and biomass processing upon the percentage composition (DM ash free) of the products produced in hydrothermal conversion of biomass. The products include oil, hydrochar, gas and an aqueous fraction; these are expressed as a percentage of the resultant product stream. Interactions have been assessed but are not included for this report.

Product fraction	Treatment											
	Vegetation community				Biomass processing				Hydrothermal conversion process			
	rus	bra	sed	*P value*	sil	pc	sed	*P value*	htc	htl	sed	*P value*
Oil	12	7	2.01	0.046	10	9	2.01	0.649	2	17	2.01	<.001
Hydrochar	43	44	9.21	1	42	45	4.53	0.445	59	28	4.53	<.001
Gas	14	9	2.19	0.121	11	12	2.19	0.431	8	15	2.19	0.018
Aqueous	31	40	5.15	0.106	38	34	5.15	0.407	31	40	5.15	0.112

Abbreviations: rus = rush; bra = bracken; sil = silage; pc = press cake; htc = hydrothermal carbonisation; htl = hydrothermal liquefaction; sed = standard error of difference.

It is possible that mass transfer into the aqueous fraction instead of the oil fraction occurred during hydrothermal conversion of bracken-derived biomass. There was no significant impact of biomass processing upon the product yields. However, in the current work there was no attempt to measure or calculate reaction rate and the gaseous phase composition was not analysed; these are research opportunities. Considering the amount of time and energy expended in a washing and pressing pre-treatment regime, hydrothermal yields are not sufficiently impacted upon by that process to render it worthwhile, although this conclusion is

based on fraction yields and not gaseous compositions. If a low mineral composition product stream is desirable (e.g. for combustion), then washed and pressed feedstocks may be preferable.

The hydrothermal conversion process had a highly significant impact upon the oil, hydrochar and gas yields, as would be expected. The hydrothermal conversion process did not impact upon the aqueous yield. There is consensus across the literature that hydrothermal liquefaction will produce more oil and gas compared to hydrothermal carbonization, and this is reflected in the current study. The stability of the aqueous yield in relation to the hydrothermal process renders the fraction a candidate for investigation as a potentially useful by-product in a bio-refinery system. The productivity of the aqueous fraction appeared to be a parameter on which downstream processes could depend. The utilization of the aqueous fraction for microbial culture could add a cultivation process into the bio-refinery model, as has been investigated by Nelson (2013).

Char production through hydrothermal carbonization of the press cake (demineralized) would be best employed for combustion fuel production. If oil production is a priority then rush-derived biomass would provide the highest yield. Hydrothermal liquefaction would be the process of choice for multiple-product streams, and hydrochar for sequestration would be best produced through carbonization of the bracken-dominated biomass.

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The yield and variation of chemical composition of cocksfoot biomass after five years of digestate application

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Abstract

Biogas production is the optimal way to utilize organic materials or energy crops and produce bioenergy. In many countries the number of biogas plants is increasing. The growth of biogas production directly influences the generation of digestate. An experiment on cocksfoot (*Dactylis glomerata* L.) fertilized with mineral fertilizer and digestate was conducted to evaluate the yield and chemical composition of cocksfoot biomass after five years of digestate application. The average results of the experiment suggest that within five years of sward use higher rates of nitrogen present in the digestate increased the biomass yield. The swards fertilized with mineral fertilizer N_{360} produced the same biomass yield as those fertilized with N_{180}. The chemical and structural biomass components varied due to the influence of type and rate of fertilizers.

Keywords: cocksfoot, digestate, chemical composition

Introduction

In order to increase bioenergy production and ensure that renewables contribute to the total energy consumption, the number of biogas plants is increasing (Angelidaki *et al.,* 2011). The main products of biogas production are methane, carbon dioxide and digestate. Biomethane may be used for generation of electricity, for direct injection into the natural gas grid, or as a fuel for vehicles (Raposo *et al.*, 2012). The use of digestate faces some problems and is a subject of considerable debate. In digestate, organic nutrients are converted and mineralized to more soluble and biologically available forms for plants (Voća *et al.*, 2005). The application of digestate as fertilizer for grasslands could be an effective way to utilize residues from biogas plants, as it can reduce the need of mineral fertilizer and increase biomass productivity (Alburquerque *et al.*, 2012). The aim of this study was to evaluate the yield and chemical composition of cocksfoot biomass after five years of digestate application.

Materials and methods

Field and laboratory experiments were carried out in Central Lithuania (55° 24' N) on an *Apicalcari - Endohypogleyic Cambisol,* light loam. The experiment was laid out in a randomized block with eight treatments and four replicates. Cocksfoot cv. Amba (developed in Denmark) was used for the experiment. No fertilization (control), two levels of mineral nitrogen fertilization (N_{180} and N_{360}) and five levels of nitrogen in digestate (N_{90}, N_{180}, N_{270}, N_{360} and N_{450}) were chosen for pure swards of cocksfoot. One half of the annual fertilizer rate was applied in early spring at the beginning of vegetation growth, the second application was made after the first cut. For each application, the quantity of digestate was calculated according to the concentration of total nitrogen in it. Total ash content was determined by ISO 3451. The concentrations of K, Na, Ca, Mg, P were estimated in sulphuric acid digestates. Concentrations of K, Na, Ca and Mg were quantified by a flame atomic absorption. P concentrations were quantified by a colouring reaction with ammonium. Total C, N and S concentrations were determined by a dry combustion method (ISO 16634). For the ADF, NDF and ADL the cell

wall detergent fractionation method (Faithfull, 2002) was used. The average chemical composition of digestate is presented in Table 1.

Table 1. The average chemical composition of digestate.

Rates mg kg^{-1}		Rates g kg^{-1} in natural material							
N-NO$_3$	N-NH$_4$	N	P	K	DM	C	Ca	Mg	pH$_{KCl}$
0.020	0.416	5.7	1.67	1.84	34	12	0.77	0.12	8.4

The swards were harvested three times per season. The first cut was taken at heading stage of cocksfoot. The differences between treatments were estimated using Fisher's Multiple Range tests. Statistical inferences were made at the 0.05 significance level.

Results and discussion

Increasing intensity of cultivation of agricultural crops results in greater productivity of biomass (Kering *et al.*, 2012). The results of the experiment suggest that cocksfoot fertilized with 180 kg ha^{-1} of nitrogen present in digestate produced a similar biomass yield to that of swards fertilized with the same rate of mineral fertilizer (Figure 1). These results are consistent with previous research, suggesting that the nutrients in digestate are already mineralized and thus can be used by plants effectively, but digestate does not surpass mineral fertilization in biomass yield formation (Fuchs *et al.*, 2008). The increase of fertilizer rate influenced intensive biomass accumulation of swards fertilized with nitrogen present in the digestate. Different results were obtained for the swards fertilized with mineral fertilizer. There was no significant difference between the swards fertilized with N$_{180}$ and N$_{360}$.
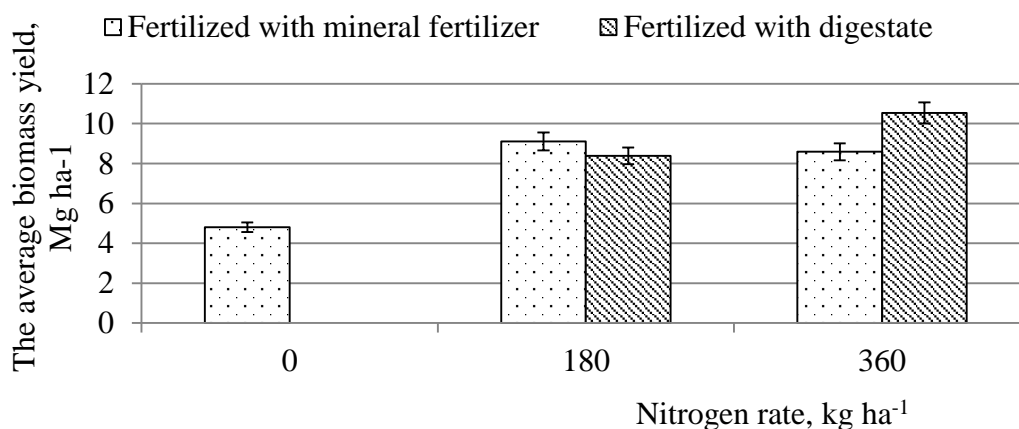
Figure 1. The average biomass yield of cocksfoot biomass within five years of sward cultivation

Because of the chemical composition of cocksfoot biomass, conventional technology is chosen. Cocksfoot biomass could be used as fodder, as a substrate for biogas production or for combustion. In our research, in cocksfoot biomass the highest variation was found for the following chemical and structural components: nitrogen, sulphur, phosphorus, calcium, chlorine, neutral detergent fibre and acid detergent fibre.

The variation of these elements was influenced not only by the type but also by the rate of nitrogen fertilizer. The swards applied with mineral fertilizer exhibited significant higher nitrogen and calcium concentrations compared to those fertilized with digestate (Table 2). An increase in fertilization rate decreased the concentration of ash in the biomass. The contents of neutral detergent fibre and acid detergent fibre were higher in the swards fertilized with digestate.

Table 2. The chemical composition of cocksfoot biomass

Fertilizer	Rate N	Rates g kg^{-1} in dry matter												
		Ash	N	S	C	P	K	Ca	Mg	Na	Cl	NDF	ADF	Lignin
Control	N0	106	18.7	2.2	424	4.4	16.5	9.9	3.9	3.8	7.1	475	339	74.8
Mineral fertilizer	N180	101	30.3	1.4	424	5.5	15.7	8.2	4.8	3.6	3.3	476	334	73.1
	N360	96	38.4	1.1	421	5.5	15.6	7.9	5.2	4.5	3.3	465	327	80.8
Digestate	N90	109	19.4	1.9	423	4.8	17.3	7.9	3.9	3.8	7.4	500	355	76.1
	N180	108	20.4	1.9	421	4.5	16.8	7.1	3.6	3.9	7.5	534	358	73.6
	N270	104	19.8	1.7	427	4.4	17.1	5.9	3.3	3.7	9.4	543	371	75.7
	N360	105	22.0	1.9	427	4.1	17.0	5.3	3.4	4.3	9.7	545	366	72.9
	N450	100	23.5	1.9	433	3.9	16.3	5.5	3.4	4.2	10.9	547	363	76.9
LSD$_{05}$		5.7	0.300	0.310	0.900	0.061	0.093	0.142	0.050	0.097	0.269		3.1	1.8

Conclusion

The use of mineral fertilizer and digestate significantly increased the dry matter yield of cocksfoot. The nitrogen fertilizer N$_{180}$ present in digestate was as efficient on cocksfoot biomass yield as mineral fertilizer nitrogen. Fertilization with digestate at a rate of N$_{360}$ significantly increased the biomass yield of cocksfoot, compared with the swards fertilized with mineral nitrogen. The chemical and structural biomass components varied due to the influence of type and rate of fertilizers.

Acknowledgments

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Evaluating sample preparation method effects on the specific methane yield of pre-and post-ensilage grass in an *in vitro* batch anaerobic digestion assay

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Abstract

The objective of this experiment was to compare the effects of two biomass preparation methods on the specific methane yields of herbages during an *in vitro* batch anaerobic digestion assay. Three contrasting pre- and post-ensilage perennial ryegrass samples were either thermally oven dried (40°C for 48 h) or non-dried (frozen to -196°C), prior to milling through a 1 mm pore sieve. These were subsequently evaluated for biomethane potential (BMP) using a small-scale *in vitro* batch digestion system. The three pre-ensilage herbages (H1, H2, H3) yielded 279, 249, 202 and 209, 211, 191 L CH_4 kg^{-1} VS_{added} with oven dried and non-dried frozen sample preparation methods, respectively. The three post-ensilage herbages (H1, H2, H3) yielded 167, 214, 182 and 214, 214, 209 L CH_4 kg^{-1} VS_{added} with oven dried and non-dried sample preparation, respectively. It is concluded that herbage samples should not be oven dried when assessing their methane potential with a small-scale *in vitro* batch digestion assay.

Keywords: grass, silage, sample preparation, anaerobic digestion

Introduction

Grass biomass can be a cost-effective feedstock for anaerobic digestion due to its potential to produce a high yield of appropriate quality biomass (McEniry *et al*., 2011) that can be ensiled to provide year-round supply of renewable energy (Plöchl and Heiermann, 2006). The biomethane potential (BMP) test is a small-scale *in vitro* batch digestion method commonly utilized to estimate the methane output of pre- and post-ensilage feedstocks (Angelidaki *et al*., 2009). A major issue for small-scale *in vitro* tests (i.e. in 100 ml to 200 ml vessels) is the need to pre-process biomass. This permits the use of relatively small (<1 g) but representative sub-samples of the biomass (Purcell *et al*., 2011). A common solution to creating a homogenous representative sub-sample is to oven dry the sample prior to milling through a 1 mm pore sieve. However, drying can reduce protein and increase fibre proportions of herbage (Alomar *et al*., 1999). This can subsequently affect the rate and extent of degradation during anaerobic digestion. Furthermore, post-ensilage feedstocks contain many volatile compounds such as volatile fatty acids (VFAs), lactic acid, ammonia and alcohols (McDonald *et al*., 1991). These compounds can be partially lost during oven drying (Kruger *et al*., 2011) thereby leading to an underestimation of the potential to produce methane. In contrast, comminuting non-dried samples (frozen to -196°C) may provide an alternative method to create a small representative sub-sample without the undesirable loss of important substrate. Thus, the aim of this experiment was to compare the relative effects of oven dried milled samples *versus* non-dried samples manually comminuted following freezing to -196°C, on cumulative methane production of pre- and post-ensilage herbage samples, using a small-scale *in vitro* batch digestion assay.

Materials and methods

Three perennial ryegrass (*Lolium perenne* L., cv. Greengold) herbages and their corresponding silages were selected from those reported by Navarro-Villa *et al.* (2012). The herbage pre-conditioning treatments aimed to provide contrasting silage fermentation conditions by using managements predisposing the silages to undergo an extensive lactic acid dominant

fermentation with minimal clostridial activity (H1), an extensive fermentation with clostridial activity (H2), and a restricted fermentation with moderate clostridial activity (H3). Both pre- and post-ensilage samples were either: (a) thermally dried in a forced air circulated oven (40°C for 48 h) and milled, or (b) non-dried, frozen to -196°C by submerging in liquid nitrogen and milled, with milling in both cases through 1 mm pore sieve.

The CH_4 yield of each sample was measured in duplicate 160 ml serum bottles over 35 days, according to the method described by McEniry and O'Kiely (2013). Briefly, bottles were incubated at 38°C with a $2_{inoculum}$:$1_{substrate}$ volatile solids (VS) ratio and a 10 g kg^{-1} organic loading rate. Headspace pressure and concentration of CH_4 in the biogas were measured. Evaluation of data included corrections for (a) inert gas dilution on day 2, (b) control (inoculum) gas production and (c) standard temperature and pressure conditions. Duplicate analytical estimates were averaged and log transformed data values were analysed with the GLIMMIX procedure (SAS 9.3) using repeated measures as a blocked split-split-plot design. The main plot comprised herbage type, the sub-plot was herbage state (i.e. pre- or post-ensilage herbage) and the sub-sub-plot was the preparation method (i.e. dried or non-dried). Replicate blocks were accounted for in the main plot. Differences between the means were compared using Tukey's adjustment for multiple comparisons.

Results and discussion

Whereas preparation method did not have an overall effect ($P>0.05$) on specific methane yield, there were interactions between preparation method and herbage state ($P<0.01$) which were of considerable importance (Table 1). Dried pre-ensilage herbage had a higher specific methane yield than non-dried pre-ensilage herbage for H1 ($P<0.05$), but the similar numerical trend for pre-ensilage herbage H2 and H3 were not significant ($P>0.05$). Thus, drying had an inconsistent effect on specific methane yield with pre-ensilage herbages.

Whereas it could be proposed that mechanical milling dry pre-ensilage herbages through a 1 mm pore sieve produces more fine particles than manually milling frozen pre-ensilage herbage through a similar sieve, and this could increase methane production during digestion, this explanation is difficult to accept in the present study because (a) the effect was not consistent across the three pre-ensilage herbages, and (b) the effect was not evident with the corresponding silages.

Drying post-ensilage herbage H1 reduced ($P<0.01$) specific methane yield, which was the opposite outcome to its corresponding pre-ensilage herbage. In contrast, the absence of an effect of drying on specific methane yield for post-ensilage herbages H2 and H3 ($P>0.05$) is similar to the outcomes with their corresponding pre-ensilage herbages. This suggests that there may have been a greater loss of volatile compounds during the drying of post-ensilage herbage from H1 than for post-ensilage herbages H2 and H3, with the loss of volatiles resulting in a reduction in the supply of digestible substrates for anaerobic digestion. Thus, oven drying samples did not have a consistent effect on specific methane yield across post-ensilage herbages H1 to H3.

Table 1. Effect of preparation method on the specific methane yield (L CH_4 kg^{-1} VS_{added}) of three pre-conditioned perennial ryegrasses and their corresponding silages. a Log transformed values (untransformed values in parentheses) b NS = not significant; *P<0.05; ***P<0.01

Herbage type (H)	Herbage state (S)	Preparation method (P)	Specific methane yield[a] (L CH_4 kg^{-1} VS_{added})
H1	Grass	Non-dried	2.32 (209)
	Grass	Dried	2.45 (279)
	Silage	Non-dried	2.32 (214)
	Silage	Dried	2.40 (167)
H2	Grass	Non-dried	2.28 (211)
	Grass	Dried	2.31 (249)
	Silage	Non-dried	2.33 (214)
	Silage	Dried	2.22 (214)
H3	Grass	Non-dried	2.33 (191)
	Grass	Dried	2.33 (202)
	Silage	Non-dried	2.32 (209)
	Silage	Dried	2.26 (182)
Standard error of the mean			
Herbage type (H)			0.03
Herbage state (S)			0.02
Preparation method (P)			0.02
S x P			0.03
Levels of significance[b]			
H			*
S			***
P			NS
S x P			***

Conclusion

Oven drying did not have a consistent effect on the specific methane yield of herbages from herbages H1 to H3 and in the case of H1 where the effect was significant these effects were in opposing directions for pre- and post-ensilage herbage states. Thus it is recommended that herbage samples for specific methane yield assessments should not be oven dried when utilizing a small-scale *in vitro* batch digestion system.

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Theme 3 posters

Area-specific bioenergy potentials from European floodplain grasslands – the DANUBENERGY project

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Abstract

Grasslands in floodplain and other wetland areas fulfil important functions considering flood protection and biodiversity conservation. The maintenance of these areas requires regular cutting and biomass removal. As the nutritive value is frequently low and the possibilities to produce high quality forage is limited due to reduced accessibility in spring, alternative options of use, such as energy production, are sought. This study investigated the energy yield potential of eight grassland sites in Central Europe in the framework of the DANUBENERGY project. Highly varying productivity between 2.6 and 11.8 t ha^{-1} a^{-1} was found. Highest net energy outputs can be expected by thermal use of the biomass.

Keywords: floodplain areas, wetland, bioenergy, solid fuel

Introduction

Floodplain grasslands are frequently characterized by valuable plant composition and, furthermore, fulfil various ecological services (Verhoeven and Setter, 2010). Considering flood protection, they play an important role as riparian grasslands are able to reduce the risk of flooding by increased infiltration capacity. In addition, the year-round plant cover minimizes the soil erosion and losses of nutrients. However, the management of these grassland sites is comparatively challenging due to particular soil conditions and, in many cases, the low nutritive value of the vegetation. Hence, these areas get increasingly abandoned in many European regions.

The project DANUBENERGY aims at developing and implementing an approach to produce solid fuel from biomass of riparian grasslands in Central Europe, taking into account technical, social and economic aspects (Wachendorf *et al.*, 2009; Blumenstein *et al.*, 2012). Eleven partners from European regions cooperate in finding solutions of site specific wetland management and grassland use for energy production. This paper investigated the biomass yield potential from eight grassland sites and assessed the potential of bioenergy production by comparing different options of energetic use.

Materials and methods

Eight grassland sites were investigated in 2013 in Italy, Slovakia, Slovenia, Austria, Czech Republic, Hungary, Germany and Poland. At each site, 3 subplots of 5×5 m were analysed for botanical composition (plant coverage) and the dry matter (DM) yield was analysed by weighing of biomass from 5 m^2 and drying of a subsample at 105 °C for 48 h.

Based on the dry matter yield, potential net energy outputs of heat and electricity were calculated by using conversion efficiency that was analysed by Bühle *et al.* (2012) for semi-natural grassland biomass. Three option of energy recovery have been considered: (1) integrated generation of solid fuel and biogas from biomass (IFBB, as a stand-alone and an add-on system to an existing energy plant with surplus heat), (2) anaerobic digestion and (3) direct combustion.

Results and discussion

Dry matter yields of the selected grassland sites ranged from 2.6 to 11.8 t DM ha^{-1} a^{-1} (Table 1). They were particularly high for typical wetland plant vegetation dominated by *Phragmites australis* and *Phalaris arundinacea*. Medium productivity was obtained by grassland vegetation with less influence of water, dominated by *Juncus effusus* and *Carex* species. Grasslands with lowest water availability showed the smallest dry matter yields.

Table 1. Dominant species, number of plant species, % coverages (cov.) of grasses, sedges/rushes, herbs and legumes and dry matter (DM) yield (± standard error) on selected sites of floodplain grasslands in Central Europe.

Site location	Dominant species	Number of plant species	Grasses cov.	Sedges/ Rushes cov.	Herbs cov.	Legumes cov.	DM yield
				%			t ha^{-1}a^{-1}
Italy	*Phragmites australis* *Phalaris arundinacea* *Typha latifolia*	8 ± 1	42.6 ± 12.6	49.8 ± 12.7	7.7 ± 2.0	-	11.8 ± 1.1
Slovakia	*Agrostis capillaris* *Holcus lanatus* *Achillea millefolium*	42 ± 4	46.7 ± 9.9	1.2 ± 0.9	46.0 ± 9.3	6.1 ± 0.4	5.7 ± 1.0
Slovenia	*Juncus effusus* *Carex* species *Alisma plantago-aquatica*	17 ± 0	2.9 ± 1.1	65.2 ± 5.8	31.9 ± 4.9	-	5.3 ± 0.5
Austria	*Agrostis stolonifera* *Phragmites australis* *Bolboschoenus maritimus*	16 ± 1	64.6 ± 13.6	19.5 ± 11.0	15.9 ± 4.6	<0.1 ± <0.1	8.6 ± 4.0
Czech Republic	*Medicago falcata* *Bromus erectus* *Centaurea scabiosa*	41 ± 8	20.8 ± 7.7	1.8 ± 1.6	34.3 ± 4.9	43.1 ± 10.2	2.8 ± 0.3
Hungary	*Festuca rubra* *Agropyron cristatum* *Achillea millefolium*	15 ± 3	74.5 ± 8.7	-	10.4 ± 3.8	15.1 ± 5.3	2.6 ± 0.5
Germany	*Carex disticha* *Phragmites australis* *Holcus lanatus*	15 ± 4	20.2 ± 8.9	87.0 ± 15.7	6.1 ± 6.2	1.7 ± 2.2	3.2 ± 0.6
Poland	*Phalaris arundinacea* *Carex acutiformis* *Carex gracilis*	30 ± 8	39.3 ± 15.7	46.9 ± 20.0	12.6 ± 3.7	1.2 ± 1.2	11.0 ± 0.9

The heterogeneity of productivity was reflected by highly varying net energy outputs, as shown in Figure 1. Highest net output could be obtained by direct combustion of the biomass. However, field drying for hay production is frequently not possible on wetland areas and, furthermore, high content of mineral nutrients requires costly combustion technologies. If the harvest takes place at a late date in autumn, the direct combustion can be the appropriate way of energy conversion, for example for *Phragmites*-dominated biomasses. In case that direct combustion of the dry biomass is not possible, the IFBB process offers an almost equivalent possibility in terms of net outputs, in particular when it is combined with a traditional biogas plant with various synergy effects. Using the grassland biomass by anaerobic digestion led to reduced net gains, what can be explained mainly by low digestibility and incomplete use of the waste heat of biogas combustion.
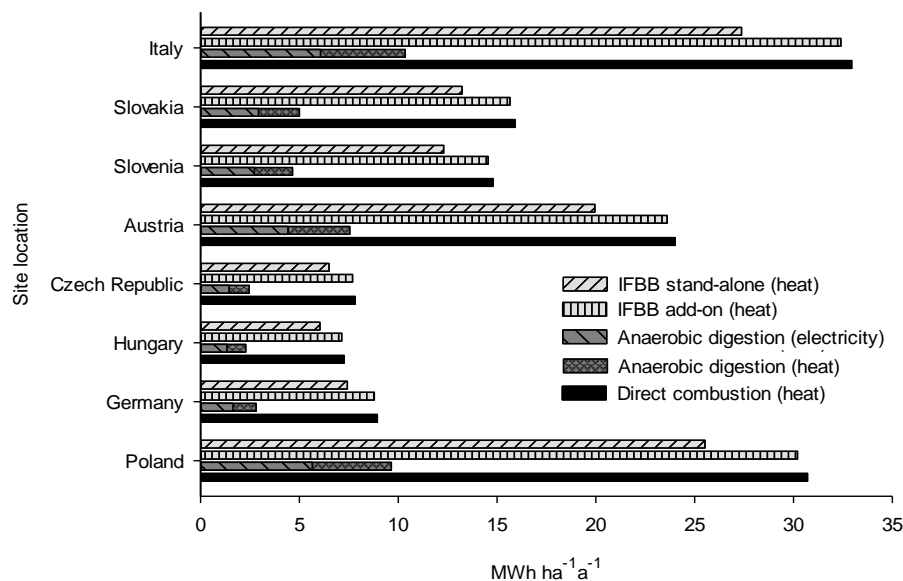
Figure 1. Net energy output (heat and electricity) of the integrated generation of solid fuel and biogas from biomass, anaerobic digestion and direct combustion on selected sites of floodplain grasslands in Central Europe.

Conclusion

In order to maintain grasslands in floodplain areas and the related functional services, e.g. biodiversity conservation and floodplain protection, energy-efficient conversion technologies are required in regions with declining forage use. Productivity and net energy output of the conversion technologies considered in this study were highly varying. With the IFBB conversion as an add-on system to existing bioenergy plants with surplus heat, highest net outputs could be obtained.

Acknowledgement

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Permanent grasslands under different management as potential source of biomass for combustion in the Czech Republic

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Abstract

The aim of this work was to evaluate different grassland managements from the viewpoint of utilization of biomass for combustion. A small-plot trial was established in the locality of Rapotín (Czech Republic) in 2003. It was managed during seven years with four levels of grassland utilization: intensive, medium intensive, low intensive and extensive. Four levels of fertilization were applied for each utilization treatment: nil-fertilization, $P_{30}K_{60}$, $N_{90}P_{30}K_{60}$, and $N_{180}P_{30}K_{60}$ (pure nutrients). Nitrogen (N) fertilization and decreasing intensity of utilization significantly increased dry matter (DM) yield up to 8.0 t ha^{-1}. The content of crude protein also increased with N fertilization up to 176.7 g kg^{-1} DM (intensively utilized treatment). The ash concentration was significantly higher in the intensive and medium intensive treatments (106.4 g kg^{-1} DM on average) than in low intensive and extensive treatments (93.3 g kg^{-1} DM on average), whereas the influence of fertilization was not significant. Excessive forage from extensively managed grasslands, with one or two late cuts and low level of fertilization, could be regarded as a favourable biofuel.

Keywords: grasslands, yield, utilization, fertilization, combustion

Introduction

In the Czech Republic the surplus of roughages in 2010 reached 995 000 t of dry matter (DM). This represents the forage for 212 000 livestock units (LU) for which there was no use, and is about one-third of forage production from permanent grasslands and an area of about 300 000 ha of grasslands in the Czech Republic (Kohoutek, 2012). A possible solution for dealing with surplus grassland forage could be its utilization as biomass for producing energy. In addition, appropriate grassland management for energy use can contribute to reduction of greenhouse gas emissions and maintain essential ecological grassland functions.

Maximizing the use of renewable resources is one of the key points of EU energy policy. The use of grassland biomass is intensively discussed for bioethanol and biogas production, and it is also considered as an alternative feedstock for solid biofuel. Each form of energetic utilization requires specific characteristics of the grassland biomass, being highly variable and depending mainly on grassland management (Goedeke *et al.*, 2008). DM yield and chemical composition can limit the suitability of grassland biomass as a fuel through environmentally damaging emissions (Tonn *et al.*, 2012). Using grassland biomass for combustion is a subject of broad research and is established in practice. Firing herbaceous biomass requires various specific adaptations of the different combustion technologies. Frydrych and Andert (2013) were engaged in alternative utilization of biomass from species-rich meadows in the Czech Republic; whole square hay bales were utilized for energy by their combustion in the unique biomass boiler (STEP Trutnov Comp). The aim of this work was to evaluate the production potential of grasslands under different management from the viewpoint of biomass utilization for combustion.

Materials and methods

A small-plot experiment (plot size 12.5 m^2) with permanent grasslands was established in Rapotín (Czech Republic) in 2003. The experimental locality was at 390 m a.s.l. During the 7-year monitoring period, average rainfall was 668 mm and temperature was 7.2 °C. The

dominant species at the beginning of the trial were: *Dactylis glomerata*, *Poa pratensis*, *Lolium perenne*, *Trifolium repens* and *Taraxacum* sect. *Ruderalia.* Grasslands were managed with four levels of intensity of utilization: I_1 = intensive (1st cut by 15 May, 4 cuts per year – cuts at 45-day intervals); I_2 = medium intensive (1st cut between 16 and 31 May, 3 cuts per year at 60-day intervals); I_3 = low intensive (1st cut between 1 and 15 June, 2 cuts per year at 90-day intervals); and I_4 = extensive (1st cut between 16 and 30 June, 1 or 2 cuts per year, second cut after 90 days). Each utilization treatment was divided to give four levels of fertilization: F_0 = no fertilization, F_{PK}= $N_0P_{30}K_{60}$; F_{PKN90}= $N_{90}P+_{30}K_{60}$, F_{PKN180}= $N_{180}+P_{30}K_{60}$ (pure nutrients). The experiment was a split plot design with four replicate blocks. Total DM yield was measured for all plots in each of the harvest years. The samples collected from the plots were analyzed for the contents of crude protein (CP), ether extract (EE), crude fibre (CF) and ash (A) by the Weende analysis. Brutto energy (BE) was predicted by the equations of Sommer *et al.* (1994). Statistical analyses were performed using ANOVA (package Statistica 10) with multiple comparisons according to Tukey ($P < 0.05$).

Results and discussion

A significant effect ($P < 0.05$) of N_{180}PK fertilization was observed, with fertilized treatments showing a higher mean value of DM yield (7.97 t ha^{-1}) than unfertilized (4.81 t ha^{-1}) (Table 1).

Table 1. Grassland total DM yield, content of nutrients and energy by different management over seven harvest years. Data is derived from a total of particular cuts in each harvest year.

Treatment	DM yield [t ha^{-1}]	CP [g kg^{-1} DM]	EE [g kg^{-1} DM]	CF [g kg^{-1} DM]	A [g kg^{-1} DM]	BE [MJ kg^{-1} DM]
I_1F_0	4.63	146.3	32.6	239.3	104.2	17.89
I_2F_0	4.70	127.5	30.0	258.1	103.1	17.78
I_3F_0	4.89	109.3	27.9	281.0	93.4	17.84
I_4F_0	5.02	102.7	25.3	303.8	94.0	17.79
I_1F_{PK}	4.63	143.2	33.5	242.7	110.7	17.78
I_2F_{PK}	4.84	127.1	32.1	260.1	109.2	17.72
I_3F_{PK}	5.17	107.3	28.1	285.6	96.1	17.80
I_4F_{PK}	5.21	104.2	26.5	301.1	97.3	17.76
I_1F_{N90PK}	6.90	157.1	33.4	234.5	106.2	17.93
I_2F_{N90PK}	6.70	141.0	32.8	256.3	108.3	17.83
I_3F_{N90PK}	7.31	120.2	27.3	296.3	94.5	17.92
I_4F_{N90PK}	7.53	118.9	26.9	307.5	97.4	17.88
I_1F_{N180PK}	7.96	176.7	34.1	235.5	103.9	18.11
I_2F_{N180PK}	7.83	159.5	32.3	259.6	105.3	18.00
I_3F_{N180PK}	8.03	142.6	28.4	295.0	87.7	18.20
I_4F_{N180PK}	8.08	128.0	27.1	306.0	86.1	18.14
ANOVA F-ratio						
Intensity of utilization	11.8**	34.4**	17.6**	36.2**	6.5**	1.20
Fertilization	589.1**	17.7**	0.7	0.1	1.3	10.2**
Year	360.6**	7.3**	3.9**	0.8	7.3**	13.2**

Intensity of utilization: I_1 = intensive, I_2 = medium intensive, I_3 = low intensive, I_4 = extensive.
Fertilization: F_0 = nil-fertilization, F_{PK}= $N_0P_{30}K_{60}$, F_{N90PK}= $N_{90}+P_{30}K_{60}$, F_{N180PK}= $N_{180}+P_{30}K_{60}$ (pure nutrients).
* $P < 0.05$; ** $P < 0.01$

With decreasing intensity of grassland utilization DM yield significantly increased; this was most apparent in unfertilized treatments. These results are in line with Kohoutek (2012).
The heating value of the sward is determined by a number of factors, whereas grassland management affects not only DM yield but also the chemical content of the biomass. Contents of ash and of several mineral elements can lead to problems during the combustion process

(Tonn *et al*., 2012). Within our study, the ash concentration in intensively and medium intensively utilized treatments differed significantly, with the higher ash concentration (106.4 g kg^{-1} DM on average) from those utilized 'low intensively' and extensively (93.3 g kg^{-1} DM on average). The reason for this may be that soil contamination was higher by harvesting the swards of lower height. Regarding the energy content we found significant effect of N$_{180}$PK fertilization, whereas the highest concentration of BE showed grasslands utilized at 'low intensively' and extensively by N$_{180}$PK fertilization (18.20 MJ kg^{-1} DM and 18.14 MJ kg^{-1} DM, respectively). We should be also aware of concentration of crude protein (CP), which increased significantly with N fertilization up to 176.7 g kg^{-1} DM (intensively utilized treatment). Nitrogen contained in the vegetation has a major role during combustion as it is the source of undesired NO$_x$ emissions (Harndorf *et al*., 2009). Taking into consideration that N concentration in the solid fuel shows a logarithmic correlation with the NO$_x$ emissions (van Loo and Koppejan, 2008) it can be stated that the risk of environmental pollution increases by excessive usage of nitrogen fertilizers.

Conclusion

On the basis of our results about DM yield and chemical properties of harvested biomass we can conclude that the grasslands utilized extensively with one or two late cuts by lower doses of fertilizers were more favourable for combustion within our study. Further research of this issue is needed.

Acknowledgement

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What is the biomethane production potential of the available grassland biomass resource in Ireland?

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Abstract

Grassland dominates the Irish landscape and is used primarily for ruminant production. In an average year, grass growth is in excess of livestock requirements and the potential exists to enlarge this excess. Four scenarios (two levels of biomass in excess of ruminant requirements; two rates of capture of the biomass excess) were assessed to elucidate the potential contribution of the available grassland resource to Ireland's 2020 renewable energy targets. Grassland can make a significant contribution to 2020 renewable energy targets in Ireland without compromising the ability of traditional agricultural systems to meet the needs of ruminant livestock, even if the latter undergo planned increases in numbers. The potential exists to provide up to 14.83, 27.43 and 25.24% of the electrical, thermal and transport energy requirement by 2020. These become more significant when double-crediting is applied, as allowed for biofuels produced from lignocellulose feedstocks in the European Union.

Keywords: Grassland, biomass, anaerobic digestion, methane, renewable energy target

Introduction

Grassland is the dominant biomass resource in Ireland and underpins most ruminant production systems. Not only is grassland plentiful, with 92% of agricultural land under the crop, but annual yields of up to 16 tonnes total solids (TS)/ha can be achieved (O'Donovan *et al*., 2011). Grass biomethane has been proposed as part of a renewable energy solution for Ireland, with biogas from the anaerobic digestion of grass being upgraded to biomethane and utilized as a transport fuel or injected into the natural gas grid (Murphy and Power, 2009; Smyth *et al*., 2009). McEniry *et al*. (2013) recently reported that under current grassland management practices there is an estimated annual grassland resource of *ca.* 1.7 million tonnes TS available in excess of livestock requirements. Furthermore, increasing nitrogen (N) fertilizer input combined with increasing the grass utilization rate of cattle has the potential to significantly increase this resource to 12.2 million tonnes TS/annum. The objective of this study was to determine the potential contribution of this available grassland resource to Ireland's 2020 renewable energy targets under specific scenario conditions.

Materials and methods

McEniry *et al*. (2013) calculated the annual grassland resource available in Ireland as the difference between current estimated grass supply and the grass requirement of the national cattle herd and sheep flock. Briefly, grass supply on various soil types was calculated as a function of annual inorganic N fertilizer input, while grass requirement was calculated based on the annual grass and grass silage TS intake requirements of each category of cattle and sheep. Using data derived from McEniry *et al*. (2013; Table 1), four contrasting scenarios (two levels of biomass in excess of ruminant requirements; two rates of capture of the biomass excess) were investigated in the current study to determine the potential contribution of this available grassland resource to Ireland's 2020 renewable energy targets.

The potential biomethane and energy yield of the available grassland resource was calculated (Tables 1 and 2). The volatile solids (VS) concentration of grass biomass was assumed to be 0.9 tonne VS/tonne TS, with a potential CH_4 yield of 400 m_n^3 CH_4/tonne VS (Nizami *et al*., 2012). The potential contribution of this available grassland resource to Ireland's renewable energy targets is expressed as a percentage of the expected energy in transport (118 Petajoule (PJ)/a), and thermal (173 PJ/a) and electrical (112 PJ/a) energy demand in Ireland in 2020 (Clancy and Scheer, 2011).

Table 1. Annual available grassland resource for anaerobic digestion (AD) and potential biomethane yield

Scenario	Grassland resource (t TS/a)[1]	Resource captured (%)	Resource for AD (t TS/a)	Resource for AD (t VS/a)[2]	Specific CH_4 yield (m_n^3 CH_4/t VS)[3]	Biomethane yield (million m_n^3/a)
1	1,669,648	10	166,964	150,268	400	60.11
2	1,669,648	30	500,894	450,805	400	180.32
3	12,203,800	10	1,220,380	1,098,342	400	439.34
4	12,203,800	30	3,661,140	3,295,026	400	1,318.01

[1] Derived from McEniry *et al*. (2013); TS = total solids. [2] VS = volatile solids; 0.9 t VS/t TS. [3] Derived from Nizami *et al*. (2012).

Table 2. Potential contribution of the available grassland resource to Ireland's 2020 renewable energy targets.

Scenario	Biomethane yield (PJ/a)[1]	Contribution to renewable energy targets (% of energy demand)[2]		
		Electrical energy (RES-E)[3]	Thermal energy (RES-H)	Energy in transport (RES-T)
1	2.16	0.68	1.25	1.15
2	6.49	2.03	3.75	3.45
3	15.82	4.94	9.14	8.41
4	47.45	14.83	27.43	25.24

[1] PJ = petajoule; energy value of biomethane = 36 megajoules/m_n^3. [2] Expected energy in transport and thermal and electrical energy demand in Ireland in 2020 is forecast to be 118, 173 and 112 PJ/a, respectively (Clancy and Scheer, 2011). [3] Assume 35 % electrical efficiency.

Results and discussion

Fossil fuels account for 94% of the energy usage in Ireland (Howley *et al*., 2012). In 2010, the Irish government set targets of 40, 12 and 10% renewable energy share in gross energy production for electrical (RES-E), thermal (RES-H) and transport energy (RES-T) by 2020, respectively (NREAP, 2010).

Under current grassland management practices there is an estimated annual grassland resource of *ca.* 1.7 million tonne TS available in excess of livestock requirements (McEniry *et al*., 2013). Table 1 outlines the potential biomethane yield generated from the capture of 10 (Scenario 1) and 30% (Scenario 2) of this resource, respectively. This has the potential to contribute 0.68 and 2.03% of Ireland's 2020 electrical energy requirements (Table 2). McEniry *et al*. (2013) also reported that there was considerable scope for increasing N fertilizer inputs to increase grassland productivity and that there was significant potential for improvement of on-farm grass utilization rates through greater adoption of currently advised grassland management programmes. Increasing N-fertilizer input combined with increasing the grass utilization rate of cattle has the potential to significantly increase the available grassland resource to 12.2 million tonne TS/a (Scenarios 3 and 4; Table 1). This has the potential to contribute up to 14.83% of Ireland's 2020 electrical energy requirements (Scenario 4; Table 2).

Grass biomethane has been proposed as the best energy crop for meeting the 2020 renewable transport energy target in Ireland (Korres *et al*., 2010). Utilization of 30% of the current available grassland resource (Scenario 2) for energy in transport has the potential to make a significant contribution to RES-T (Table 2), considering the EU Renewable Energy Directive

allows a double credit for biofuels produced from lignocellulosic feedstocks (i.e. 6.9% contribution to RES-T in 2020 for Scenario 2).

Conclusion

The available grassland biomass resource in Ireland can make a significant contribution to the 2020 renewable energy targets. Furthermore, considerable potential exists to make a significant (up to 14.83% renewable energy share; Scenario 4) contribution to RES-E or to surpass the targets set by RES-T (up to 16.82 and 50.48% renewable energy share for Scenarios 3 and 4, respectively), through changes to grassland management practices. Of particular significance is that this may be achieved without competing with traditional agricultural production systems.

Acknowledgements

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Evaluation of biomass yield of energy crops using waste products as fertilizers

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Abstract

Biomass from perennial grasslands might be a valuable renewable resource. At the same time the successful management of large amounts of waste products is very important. Field experiments were carried out in the central part of Latvia (56° 42' N and 25° 08' E latitude) to estimate reed canary grass (*Phalaris arundinacea* L.), festulolium (×*Festulolium pabulare*) and galega (*Galega orientalis* Lam.) dry matter (DM) yield in two years of sward use. The fertilization treatments were control (no fertilization), sewage sludge, biogas digestate and wood ash. Doses of fertilization were calculated in order to provide similar amount of plant-available potassium (K) from each fertilizer. Trial results showed a significant dry matter yield dependence on the grass species and the applied fertilizer. The significant dry matter yield increase on average for two years of use at two cutting regimes for grasses and galega provided using sewage sludge (1.59 t ha^{-1} and 1.41 t ha^{-1} respectively) and digestate (1.27 t ha^{-1} and 1.34 t ha^{-1} respectively).

Keywords: fertilization, perennial grasses, biomass yield, by-products, renewable resources

Introduction

Grasses and forage legumes used for biofuel production can provide a number of ecosystem services. Grassland can fix atmospheric nitrogen, improve soil texture and increase biodiversity at the field and landscape level (Prade *et al*., 2013). Grasslands, compared to other crops for agro-fuel production, can be produced on marginal agricultural land; they do not require large amounts of fertilizers and pesticides (Kryzeviciene *et al*., 2008; Peeters, 2008). Biomass cultivation could become an alternative to those farmers in Latvia whose agricultural land is not suitable for cultivation of cereals. The use of renewable energy resources will reduce the dependence on imports of fossil fuels (Adamovics *et al*., 2011). Reed canary grass, festulolium and galega are well known as productive species suitable for biogas or fuel-pellet production (Rancane *et al*., 2013).

There is an important potential to use different by-products for grassland fertilization. Returning waste products to agricultural land by the application of waste-based fertilizer products is way to solve the problem of disposal of waste products and promote nutrient recycling (Bougnom *et al*., 2012; Brod *et al*., 2012).

The objective of the current research was to study the effect of different bio-energy products and municipal waste products (digestate, wood ash and sewage sludge) used as fertilizers, the choice of grass species, and the intensity of management on grass and legume productivity.

Materials and methods

Field trials were performed at the Research Institute of Agriculture in Skriveri on Phaeozems (soil pH$_{KCl}$ 6.1, plant available phosphorus (P$_2$O$_5$) – 277.1 mg kg^{-1}, potassium (K$_2$O) – 136.8 mg kg^{-1}, organic matter content 23 g kg^{-1}). The trials were sown in July 2011 without a cover crop, in randomized block design with four replications, and a 20 m^2 harvested plot size. Three potential energy crop grass species were investigated for dry matter yield: reed canary grass, festulolium (tall fescue type) and galega. Two intensities of management were applied: 3 cuts per vegetation period and 1 cut in October. The following fertilization treatments were used: control (no fertilization), sewage sludge, biogas digestate and wood ash. Doses of fertilizers

were calculated in order to provide a similar amount of plant-available potassium (K) from digestate and wood ash (Table 1).

Table 1. The nutrient content and applied amount of fertilizers

Fertilizer	Nutrient content in the fertilizers dry matter, g kg⁻¹			Applied nutrient per year, kg ha⁻¹		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Control	0	0	0	0	0	0
Wood ash	0.73	14.7	88.2	1	20	120
Digestate	29.2	22.4	53.8	65	50	120
Sewage sludge	46.3	71.0	2.5	150	230	8

The low content of potassium in sewage sludge did not allow the 120 kg ha⁻¹ input of K_2O per year to be reached; therefore calculation of the applied sewage sludge dose was conducted according to the input of medium-low nitrogen (N) dose (N150). Dry matter yield were determined for two years of sward use (2012 and 2013). The experimental data were statistically analysed using by two-way analysis of variance with species and fertilizer as factors, and the difference among means was detected by LSD at the 0.05 probability level (Excel for Windows 2003).

Results and discussion

The average dry matter yields of the two grasses and one legume were considered as satisfactory in the first (6.76 t ha⁻¹) and moderate in the second (5.48 t ha⁻¹) production year. The lowest yield in the second year can be explained by meteorological conditions – very late spring and an insufficient amount of precipitation and unfavourable distribution of rainfall in summer Using a 3-cut cutting frequency, there was a significant DM yield increase, on average for the two years, provided by sewage sludge (1.65 t ha⁻¹) and digestate (1.32 t ha⁻¹) for all investigated species (Table 2).

Table 2. Average dry matter yield for two years of sward use at frequent cutting, t ha⁻¹

Species (F_B)	Fertilizer (F_A)				Mean
	Control	Wood ash	Sewage sludge	Digestate	
Phalaris arundinacea	4.64	5.16	6.18	6.30	5.57
Festulolium	4.32	4.98	5.99	5.42	5.17
Galega orientalis	6.71	7.36	8.46	7.88	7.60
Mean	5.22	5.83	6.87	6.54	

$LSD_{0.05}$ for DM yield: F_A= 0.89; F_B= 0.77; F_{AB}= 1.54

The highest average DM yield at the frequent cutting regime was given by galega, as this N-fixing legume had good regrowth after cuts. The average festulolium and reed canary grass DM yields did not differ significantly.

The same effect of fertilization was observed with cutting once in a season – at crop senescence. Significant DM yield increases were provided sewage sludge (1.40 t ha⁻¹) and digestate (1.27 t ha⁻¹) (Table 3).

Table 3. Average dry matter yield for two years of sward use at autumn cutting, t ha^{-1}

Species (F_B)	Fertilizer (F_A)				Mean
	Control	Wood ash	Sewage sludge	Digestate	
Phalaris arundinacea	6.13	6.04	7.21	7.67	6.76
Festulolium	4.91	5.24	6.96	5.66	5.69
Galega orientalis	5.14	5.64	6.20	6.64	5.91
Mean	5.39	5.64	6.79	6.66	

LSD$_{0.05}$ for DM yield: F_A= 0.82; F_B= 0.71; F_{AB}= 1.42

Analysis of variance showed that the influence of species factor on DM yields was significant. The highest average DM yield was given by reed canary grass under at the management with one late cut. By mowing galega once per season in autumn a relatively low DM yield was obtained, which can be explained by partial plant defoliation.

The DM yield increase achieved by applying wood ash was not significant for either of the intensities of management. On average for two production years, intensity of management did not provide significant differences of DM yield.

Conclusion

The productivity of perennial grass biomass was dependent on the type of applied fertilizer and the species. On average for two production years, the highest dry matter yield increase was provided by fertilizing with sewage sludge. For galega, the highest average DM yield was produced using a 3-cut cutting frequency, but for reed canary grass the highest average DM yield was at the management with one late cut.

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Utilization of reed canary grass in pellet production

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Abstract

Pellets are renewable and environmentally friendly biofuel. Perennial grasses may be one of the raw materials used for pellet production. In some countries they are the main source of bioenergy, since their longevity also ensures substantial and stable production of biomass under less favourable climatic conditions. The research aims at finding out the suitability of reed canary grass (RCG) for production of pellets by studying the influence of various factors (e.g., component proportions in pellet, fertilizers applied) on suitability for combustion of the pellets. The study also investigated effect that the amount of nitrogen fertilizers applied on crops had on the pellet quality and suitability for combustion. Analysis of the combustion suitability showed the highest reed canary grass biomass indicators for component proportion 1/3 (RCG/wood). Therefore, reed canary grass may be cultivated as an alternative energy crop that may be utilized for pellet production.

Keywords: reed canary grass, pellets, combustion ability

Introduction

In some countries perennial grasses are the main source of bioenergy, since their longevity ensures substantial and stable production of biomass under less favourable climatic conditions. Grasses tend to occupy large share of the agricultural area and are essential for the agricultural production sector. Pellets are an environmentally friendly biofuel, as they may be produced from renewable sources. This fuel is produced from dried woodworking waste − sawdust, shavings, bark, twigs, branches *etc*., but pellets may also be made from various grasses, mixtures thereof, natural grass from meadows, or reed (Lewandowski *et al*., 2003; Adamovics *et al*., 2007; Adamovics *et al*., 2009; Strasil, 2012). The energy released by 1 kg of wood pellets equals that from 0.5 l of solid fuel. It has been found that substitution of heavy fuel oil with pellets results in a reduction of approximately 4.8 t CO_2 released annually, while substitution of gas in 2.5 t less. The transport and storage of pellets are not hazardous to the environment. Ash produced from burning can usually be used as a source of fertilizers, as its content of chemical elements does not exceed required standards (Adamovics *et al*., 2007). RCG biomass is currently considered as one alternative raw material source that can be used for pellet production. This grass plant has characteristics of good persistence under local climatic conditions and has high biomass yield per hectare. The aims of this research were to determine the suitability of reed canary grass for production of pellets by studying the influence left by various factors (e.g., component proportions in pellet, fertilizers applied) on suitability for combustion of the pellets.

Materials and methods

The Research objects were reed canary grass (*Phalaris arundinacea* L.) and energy wood species: osier (*Salix viminalis* L.) and poplar (*Populus tremula* L.). The energy woods (osier (sp 'Tordis') and poplar were grown at the Vezaiciai agricultural research institute in Lithuania. Pellets were produced by mixing reed canary grass (variety 'Marathon') with osier or poplar in various dry matter proportions − 1/3, 1/1, or 3/1. The pellets were made from 100% natural ingredients − chopped wood (osier or poplar) and chopped RCG biomass. Each biomass sample was tested three times. Within the pellet manufacturing process, plant biomass was chopped and ground in the laboratory mill (ЭМ-3А УХЛ 4.2) and afterwards the powder acquired was formed into a pellet with the hand press ('IKA WERKE'). Combustion ability in the samples

was measured with the help of a calorimeter (capsule 'IKA C 5003') based on LST CEN/TS 14918:2006 standard; lignin content was in compliance with the LVS EN ISO 13906: 2008, and ash content, with ISO 5984: 2002 standards.

The trial data were processed using correlation, regression and variance analyses (ANOVA) and descriptive statistics with Microsoft Excel for Windows 2000 (Arhipova and Balina, 2006). The means are presented with their LSD test.

Results and discussion

Combustion ability is the main feature of fuel as it indicates the its efficiency (Friedl *et al*., 2005). Analysis of combustion ability of pellets made from reed canary grass and osier at various proportions depending on amount of nitrogen fertilizer applied showed the best combustion indicators for samples not treated with nitrogen. The best combustion ability was found in pellets made from reed canary grass and osier in combination of 1/3. Samples that were previously treated with nitrogen fertilizer indicated a combustion ability (energy value) of 18.57 MJ kg^{-1}, whereas ones from RCG not treated with fertilizer were 18.69 MJ kg^{-1} (Figure 1).
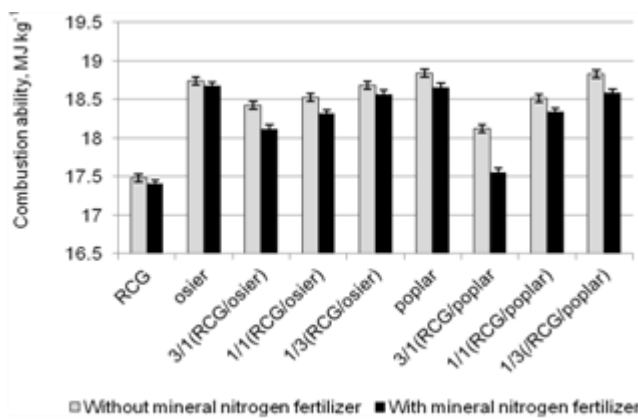
Figure 1. Combustion ability of reed canary grass pellets depending on amount of nitrogen fertilizer applied

The highest combustion ability of pellets made from reed canary grass and poplar was in the proportion 1/3, and their energy value comprised 18.59 MJ kg^{-1} with nitrogen fertilizer, and 18.83 MJ kg^{-1} without fertilizer. Our research did not show that nitrogen fertilizers have a notable influence on combustion ability; therefore Figure 2 summarizes pellet combustion ability, average lignin and ash content as well as component proportions.
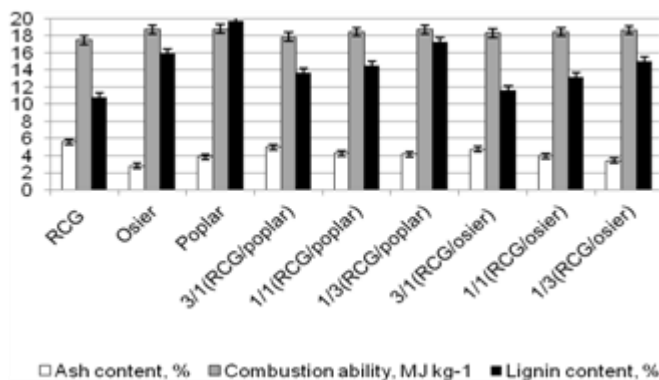
Figure 2. Combustion ability, lignin and ash content in pellets made from biomass in various proportions

Reed canary grass biomass has high ash content (5.59%), but it is lower in pellets made in proportion 1/3 (RCG/wood); moreover both osier and poplar indicated relatively lower ash contents of 3.43% and 4.18%, respectively.

The lignin content in reed canary grass biomass accounts for 10.78%, whereas higher lignin content was observed in samples made from reed canary grass and wood in proportion 1/3: 14.97% with osier and 17.28% with poplar.

The research showed that pellets made solely from reed canary grass biomass have lower combustion ability than pellets made from reed canary grass together with osier and poplar. The highest reed canary grass combustion ability indicators were recorded for combination 1/3: 18.71MJ kg^{-1} for pellets RCG/osier and 18.63MJ kg^{-1} for RCG/poplar pellets.

Conclusions

Reed canary grass is suitable for pellet production. Nevertheless, it is advisable to mix it in proportion of 1/3 with wood. Average combustion ability of pellets made from biomasses in various proportions varies between 18.53 MJ kg^{-1} in samples not treated with nitrogen, and 18.34 MJ kg^{-1} in samples on which nitrogen was applied. The highest combustion ability was recorded in pellets made from reed canary grass and wood in combination 1/3. Nitrogen fertilizers leave a slight influence on pellet quality and combustion ability. Characteristics and chemical composition of reed canary grass are similar to timber, but when burnt, pellets of this grass produce more ash, and therefore when producing pellets it should be mixed with sawdust or woodchips.

Acknowledgements

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Can specific methane yield of perennial ryegrass be reliably predicted?

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Abstract

We investigated the potential of two approaches to predict the specific methane yield (SMY; l_N CH_4 $(kg\ OM)^{-1}$) of perennial ryegrass. Multiple linear regression (MLR) was found inappropriate, explaining at best 50% of the variation in SMY. Near-infrared reflectance spectroscopy (NIRS) seems a more promising approach, as indicated by prediction errors of 11.7 and 15.9 (l_N CH_4 $(kg\ OM)^{-1}$) and coefficients of determination of 0.82 and 0.67 for the calibration and validation, respectively.

Keywords: *Lolium perenne*, specific methane yield, forage quality, multiple linear regression, NIRS

Introduction

Methane production by anaerobic digestion is discussed as an alternative for grassland not used any longer by livestock. Furthermore, ley-arable systems might reduce potential adverse environmental impact of biogas substrate production, which is often dominated by continuous maize. The evaluation of grassland methane yield, however, still requires the use of labor- and cost-intensive batch assays. The present study aimed to investigate whether SMY of perennial ryegrass can be predicted from forage quality traits or by NIRS.

Materials and methods

The study was based on data collected in a 2-year (2009-2010) grassland trial conducted on a heavy clay soil (40% clay, gleyic Fluvisol (calcaric), pH 7.2) close to the west coast of Schleswig-Holstein, northern Germany. Two *Lolium perenne* cultivars differing in heading date (Trend, 4n, mid-early; Twymax, 4n, late) were grown in a 3- and 4-cut system. Additional samples were obtained from an adjacent cropping system experiment (2009-2010), where cv. Trend was grown in a 4-cut system. In both trials, mineral N fertilizer was applied as calcium ammonium nitrate at a level of 360 kg N ha^{-1}. The contents of N, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), crude fat (XL), water-soluble carbohydrates (WSC), crude ash (XA), as well as enzyme soluble organic matter (ESOM), digestibility of organic matter (DOM) and metabolizable energy content (ME) were estimated by NIRS (NIRS-System 5000 monochromator, Foss NIRSystems, USA; WinISI II, Infrasoft International, USA). Specific methane yield (l_N CH_4 $(kg\ OM)^{-1}$) was determined by a batch assay. For this purpose, fresh crop samples were chopped to 2 cm length and subsequently stored at -20 °C. The samples were slowly defrosted before analysis. The batch test was conducted in two replicates using 2 L glass vessels filled with 1.9 L of inoculum and a quantity of 3 g crop substrate per litre inoculum. The inoculum consisted of a mixture (1:1) of sludge obtained from a laboratory fermenter and digested sludge from a regional sewage plant. Vessels were incubated at 38 °C and the batch assays were continued until biogas emission became negligible (< 1% per day), at least for 30 days. Each run included one sample of a pure inoculum and one microcrystalline cellulose sample. The methane amount was determined with a gas chromatograph (Varian 3800). For further data analysis, SMY was adjusted to norm conditions (273 K, 1013 mbar). In a first approach, a MLR model was applied (lme-function in the R-package nlme) to determine how accurate SMY can be estimated by forage quality traits. To this end, ADL, HCEL, CELL, XP, XL, XA and WSC served as predictors, by assuming year and replicate as random. Aggregating quality traits, e.g. energy concentration, were not

considered to avoid multicollinearity. In a second approach, the ability of NIRS for predicting SMY was analysed. From the pooled data sets of the grassland and cropping system experiments, a calibration subset of 110 out of 183 samples was selected to represent the whole spectral and chemical variability, using the H-value as selection criterion. The remaining 73 samples provided the validation subset.

Results and discussion

The SMY ranged between 283 and 422 l_N CH_4 $(kg\ OM)^{-1}$. The analysis of the relationships among quality traits revealed plausible correlations among all quality traits (data not shown). Slightly negative but significant relationships were detected between SMY and N content, cell wall fractions, and crude fat content. In contrast, WSC, energy content, ESOM and DOM seem to result in increased SMY. These results are partly in contrast to previous research (Kandel *et al.*, 2013a, b; McEniry and O'Kiely, 2013). Overall, none of the single quality traits explained the variation of SMY to a sufficiently high degree.
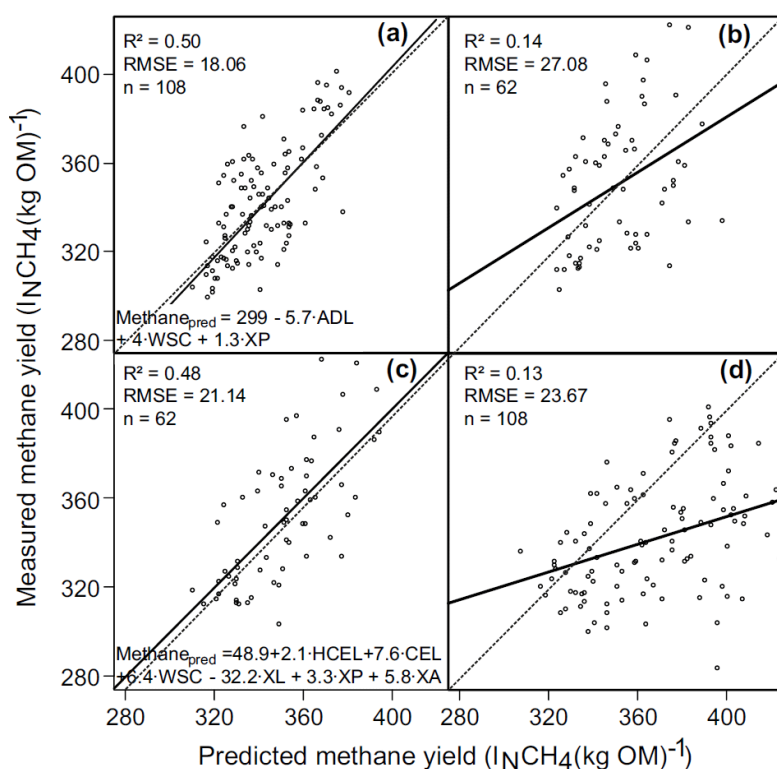
Figure 1. Results of MLR, conducted separately for samples collected in the grassland (a) or cropping systems experiment (c). Regression functions obtained for (a) were validated with the cropping systems data set (b) and functions obtained for the cropping system experiment (c) were validated with the grassland data set (d). ADL: acid detergent lignin (% DM), CEL: cellulose (% DM), HCEL: hemicellulose (% DM), XA: crude ash (% DM), WSC: water-soluble carbohydrates (% DM), and XP: crude protein (% DM).

The MLR was conducted (i) separately for the grassland experiment and the samples originating from the cropping systems trial for calibration and validation purposes, (ii) with additional differentiation by the different cuts, and (iii) for the pooled data set of both experiments. Altogether, none of the approaches led to a statistical model that could explain the variation in SMY by more than 50%. This is exemplified in Figure 1 for a separate analysis of both data subsets (grassland, cropping system). In agreement, Raju *et al.* (2011) and Kandel *et al.* (2013a) investigating SMY of grasses or plant parts did not find meaningful prediction functions explaining more than 40 to 57% of SMY variation.

The difficulties associated with MLR models can be overcome when predicting SMY from NIRS spectra (Table 1). The calibration equation was acceptable, as indicated by an R^2 of 0.81

and a low standard error of calibration (SEC) of 11.7 l_N CH_4 (kg OM)$^{-1}$. When comparing the variation of SMY found in our data set with the results of the multiple linear regression approach and the precision of the batch assay (Heuwinkel *et al.*, 2009) the calibration seems promising. Independent validation of SMY was classified as reliable, as indicated by a standard error of prediction (SEP) of 15.9 l_N CH_4 (kg OM)$^{-1}$ and a R^2 of 0.67. Similar to our study, Raju *et al.* (2011) and Kandel *et al.* (2013b) reported a better performance of NIRS than of models indirectly estimating grass SMY from forage quality traits. Apparently, SMY is affected by a large number of constituents and corresponding associations, which are reflected in NIRS spectra, but cannot be reliably quantified in simpler multiple regression approaches.

Table 1. Calibration and validation statistics for the prediction of specific methane yield (l_N CH_4 (kg OM)$^{-1}$) by near infrared reflectance spectroscopy. Range, mean and standard deviation (SD) refer to the laboratory-determined values of the calibration and validation subsets. SEC: standard error of calibration, SECV: standard error of cross validation, SEP: standard error of prediction.

N	Range	Mean	SD	Mathematical treatment [1,2,3,4]	SEC	SECV	SEP	R^2
Calibration set								
110	283.5-421.9	348.7	24.3	2,5,5,1	11.7	15.6	--	0.81
Validation set								
73	301.9-392.0	345.5	22.1	2,5,5,1	--	--	15.9	0.67

[1] number of derivate of the log (1/*R*) spectrum; [2] segment of the gap over which the derivative was calculated; [3] number of data points used during first smoothing of the spectrum; [4] number of data points used during second smoothing of the spectrum

Conclusion

NIRS seems a promising tool for predicting ryegrass SMY, as our study indicates. However, further improvement will be necessary before it can be used as a tool for screening breeding materials or for optimizing feedstock provision for biogas plants. This may, in part, be due to the fact that spectra were recorded for dried and ground samples, whereas in the batch assay, chopped, frozen samples were used.

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Phytoestrogen content in clover (*Trifolium* spp.) and in grass stands depending on treatment and storage

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Abstract

Beneficial as well as negative effects of phytoestrogens (PEs) on foodstuffs and fodder of plant origin have been studied. In this work, PE levels were examined over 2 years in red clover (*Trifolium pratense*), zigzag clover (*T. medium*) and their hybrid, as well as in haylage from two locations in the Czech Republic produced from the first cutting of grass on perennial grass stands. A statistically significant difference in PE content was found between the two species of clover. Plants in the hybrid population contained a statistically inconclusive difference in PE content in comparison with *T. pratense* and a lesser PE content than *T. medium*. In high- and low-quality haylage samples, a significantly higher PE content was found in samples from the Závišice location (2011 and 2012 harvest), which may be due to the difference in individual species representations in the fodders at the given locations.

Keywords: phytoestrogens, *Trifolium pratense*, *Trifolium medium*, UHPLC-MS/MS

Introduction

Phytoestrogens (PEs) are biologically active chemical compounds of plant origin which display effects similar to those of oestrogen sex hormones (Velíšek *et al*., 2009). In recent years, interest in these substances has grown with a view to their possibly beneficial but also possibly negative effects on humans and animals. The main and most significant sources of PEs are considered to be pulses (in particular soya beans (*Glycine max*) and fodder crops (red clover (*Trifolium pratense*) and alfalfa (*Medicago sativa*)) which are used as feedstuffs (Kuhnle *et al.,* 2008). The PEs most represented in fodder crops are biochanin A and formononetin while those in pulses are daidzein, genistein and glycitein (Beck *et al*., 2005). In mammals, PEs are metabolized to products which generally display higher oestrogen activity than those original forms.

The aim of this two-year study was to examine the PE profile of a new hybrid population, which was bred through interspecific hybridisation of red clover and zigzag clover (*T. medium*) (Jakešová *et al*., 2011). A further aim was to evaluate PE content in high- and low-quality haylage.

Materials and methods

Breeding material acquired by *T. pratense* x *T. medium* interspecific hybridization was tested for content of daidzein, genistein, formononetin and biochanin A in 2011 and 2012. The 'Amos' variety and *T. medium* were parental genotypes used for comparison. The haylages were produced from the first cutting of perennial grass stands at Lukov and Závišice in the Czech Republic during 2011 and 2012. Haylage was produced and stored in the form of plastic-wrapped hay bales. Low-quality haylage was prepared by artificially damaging the protective plastic film covering the hay bales in several places with the tines of a pitchfork (simulating damage from branches) and by slicing it with a knife (simulating greater damage). Approximately once per week the damaged bale was sprinkled with 10 L of water (simulating

rainwater leaking into the damaged bale). Total PE content in low-quality haylage was examined and compared with high-quality haylage.

To isolate PEs from the matrix, direct extraction (to determine free PEs) and acid-based hydrolysis (to determine total PEs) were used. To determine the PEs, an optimized and validated method of ultra-performance liquid chromatography in combination with triple quadrupole mass spectrometry (ULPC-MS/MS) was used. Separation was done on an Acquity BEH C18 (50 x 2.1 mm; 1.7 μm) analytical column.

Results and discussion

Differences were found in the PE profile and content among the individual tested clover materials. A statistically significant difference in PE content was found between the two species of clover (Figure 1).
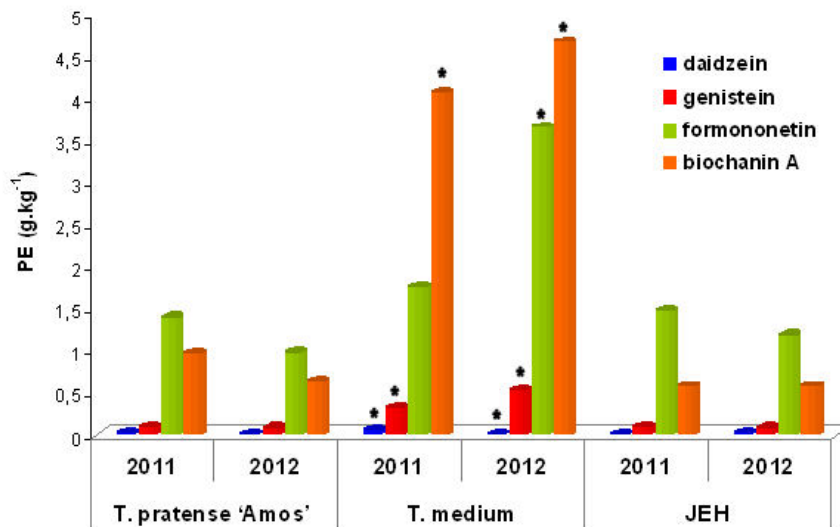
Figure 1. Comparison of phytoestrogen (PE) content in *Trifolium pratense*, *T. medium* and their hybrid (JEH) in the two-year experiment. * Statistically significant difference.

Plants in the hybrid (JEH) population contained a statistically inconclusive difference in PE content in comparison with *T. pratense* and a lower PE content than did *T. medium*. A higher content of biochanin A was observed in *T. medium* than in the hybrid JEH plants, and in the latter there was, by contrast, a higher content of formononetin.

Breeding material founded on *T. pratense* x *T. medium* hybrids is a rich source of genetic variability for *T. pratense*. That material's distinct DNA content had been analysed by flow cytometry and cytology (Řepková *et al.*, 2012) and was characterized by increased variability in morphological and agronomic traits (Jakešová *et al.*, 2011). The newly cultivated plants with the JEH designation were used to breed the new variety 'Pramedi'. The 'Amos' variety was used to stabilise the genomes of the hybrids by repeated open pollination with the JEH genotypes. The Czech Plant Variety Office granted rights for the new variety in 2013 (variety number TPM14855 and variety code 5082339).

A comparison of total PE content in high-quality (HQH) and low-quality (LQH) haylages (each material was always analysed from the upper layer, middle layer and at the centre of the bale) showed a slightly higher PE content in HQH samples from Závišice in 2012. No significant difference was observed in PE content between HQH and LQH from Lukov in either harvest (2011 or 2012). A higher PE content was noticed in 2012 for both HQH and LQH samples in both studied areas (Figure 2).
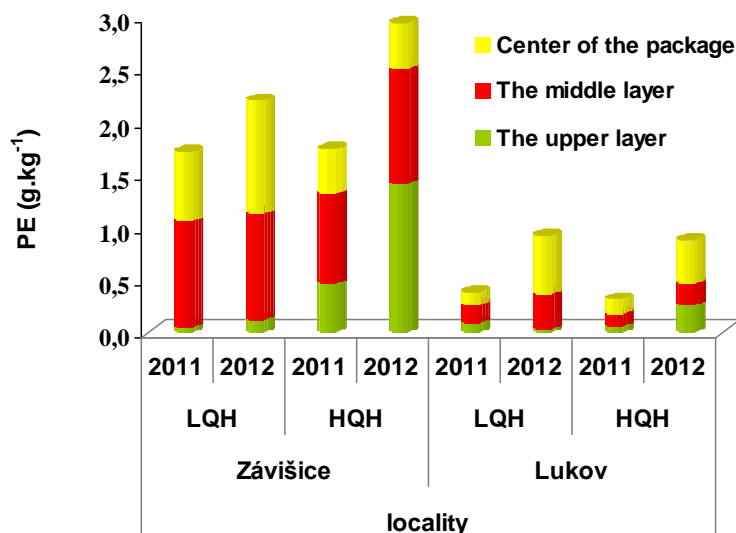
Figure 2. Comparison of total PE content in high-quality (HQH) and low-quality (LQH) haylages from Závišice and Lukov in the two-year experiment.

Comparison of PE content in individual layers of the bales demonstrates that the middle layer of the bale has the richest PE content in both HQH and LQH. Only in one instance was PE content higher in the upper layer of the bale, namely in the LQH sample from Závišice from 2012. The overall higher PE content in haylage from Závišice is primarily owing to the higher representation of red clover in the grass stand there (about 70%).

Conclusions

A statistically significant difference in phytoestrogen content was found between the individual species of clover. Plants in the hybrid JEH population contained a statistically inconclusive difference in phytoestrogen content in comparison with *T. pratense*. In high- and low-quality haylage samples, a significantly higher PE content was found in the sample from Závišice (2011 and 2012 harvests).

Acknowledgements

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Demand for K and P in reed canary grass (*Phalaris arundinacea*) during the harvest years

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Abstract

Reed canary grass (RCG) is a tall perennial grass that can be used as fuel in boilers if harvested in a delayed harvest system. To increase the crop's ability to compete with forest fuels, there is a need for cropping cost reductions. We did fertilization experiments during two harvest years at two sites in Sweden, one in the north and one in the south, to determine if P and K fertilization can be reduced and to determine the effect of recycling of RCG ash as a fertilizer. All treatments were given 100 kg N per ha. Both sites were moderately low in plant available P and K. There was no increase in RCG yield by P, K or RCG ash fertilization. However, significant differences in the P and K concentrations in the crop and in plant available P and K in the soil were observed. We conclude that even in relatively P and K poor soils, P and K fertilization of RCG can be omitted or replaced with recycling of RCG ash without any negative short-term effects on yields.

Keywords: *Phalaris arundinacea*, yield, PK fertilization, ash fertilization

Introduction

Tall perennial grasses have been identified as potential second-generation energy crops. They can be used as fuel in electricity and heat production or as raw material for ethanol and gas production. A system with cutting in late autumn, storage in windrows in the field during winter, and harvest in spring after aeration of the windrows (delayed harvest) was developed in Sweden in the early 2000s (Larsson *et al.*, 2006). The costs for growing of RCG are lower than for most other crops since the fertilization costs are low and, if the crop is successfully established, it can be sustained over 15 years (Pahkala, 2007) so for each harvest year the establishment costs are low. Despite low growing costs, however, the total production costs are still too high for RCG to compete with fuels that are by-products from forestry in Sweden. Thus the costs need to be reduced, and one way can be to improve the fertilizer recommendations for P and K and also recycle nutrients in the ash from combustion of RCG to avoid ash disposal costs. RCG has a good capacity to relocate N, P and K from the shoot to the rhizomes during late autumn and reuse it spring growth the following year (Xiong *et al.*, 2009) and it also is well known for efficient nutrient uptake. Thus it might be possible to reduce the P and K fertilization rates after the first two years when the rhizome system is established. The aim of this study is to determine the short-term requirement (2 harvest years) of P and K for RCG in a delayed harvest system on soils with low P status. We also aim to determine if this requirement can be satisfied by fertilization with RCG ash.

Materials and methods

Field experiments were performed at two RCG fields at farms, selected due to low concentrations of plant available P and K in both top soil and sub soil. The site in southern Sweden was Runtorp (56° 36' N 15° 58' E) close to Kalmar. The soil is a sandy loam. The RCG was sown in June 2011 and fertilized with 20 t ha^{-1} biogas residue prior to sowing. The experiment started in May 2012 at the start of the first production year for the crop. The site in northern Sweden, Röbäcksmyran (63° 48' N 20° 9' E) close to Umeå, is a drained former shallow peatland with the depth of peat approximately equal to the depth of the top soil (20-30

cm). The soil is humus-rich loamy sand with sandy subsoil. This field was sown in June 2007 and was fertilized with horse manure prior to sowing. In the following years it was only fertilized with 80 kg N ha^{-1}, not with P or K. The start of this experiment was in June 2012.

The experiments had a randomized block design with four replicate blocks. Each block had seven plots (3×9 m) with different treatments. All treatments received a basic fertilization of 100 kg N ha^{-1} as Axan NS 27-4. In addition, in the first year, RCG ash (3000 kg ha^{-1} with 34 kg P and 104 kg K ha^{-1}) was spread one treatment. The ash was non-hardened (i.e. not treated by moistening before storage). The other treatments were two levels of P (34 and 17 kg ha^{-1}), two levels of K (100 and 50 kg ha^{-1}), a control fertilized with the recommended level of P and K for forage grass (20 kg P and 130 kg K kg ha^{-1}), and a control without P and K fertilization. The second year, the ash and P treatments received only N, while the other treatments were the same as in the first year. All fertilizations were made at the start of the growing seasons.

Soil samples were taken from the topsoil (0-20 cm) and the subsoil (40-60 cm) using an auger with 3.5 mm diameter for the top soil and 2.9 mm diameter for the subsoil. Five samples from each level and block were pooled to a composite sample at the start of the experiment. After harvest 2013, all plots in the unfertilized control treatment, the ash treatment and the PK fertilized control were sampled in the same way to one composite sample per plot. Plant-available soil nutrients were estimated by extraction with 0.01 mol l^{-1} ammonium lactate and 0.40 mol l^{-1} acetic acid (AL method, Swedish standard, SS 028310).

The plots were harvested each year with Haldrup harvesters in November in the south and in October in the north. The harvested plots were 1.5×6 m in the middle of the plots. The dried samples were milled on a hammer mill with 1 mm sieve before chemical analysis. The mineral concentrations of the milled crop 2013 were analysed after digestion with HNO$_3$ using an Induced Coupled Plasma.

The results were analysed by balanced design ANOVA with treatment as fixed effect and block as random effect using NCSS 8 (Hintze, 2012). Each experiment was analysed separately. The soil measurements for top soil and sub soil were analysed separately.

Results and discussion

Yields did not differ significantly between the treatments at any of the sites. At the southern site, Runstorp, the mean yield in November was 10.0 t ha^{-1} in 2012 and 9.7 t ha^{-1} in 2013. At the northern site, Röbäcksmyran yields were lower: The mean yield in October 2012 was 6.1 t ha^{-1} in 2012 and 7.5 t ha^{-1} in 2013. The yields were similar to other Swedish studies with harvest in late autumn (Landström *et al.*, 1996). The summer of 2012 was a comparatively rainy summer in southern Sweden and this should have been advantageous for RCG. Its growth is often more limited by water than by N availability (Kätterer and Andren, 1999). Mineral concentrations in the autumn harvest differed more between the treatments at Röbäcksmyran than in Runstorp. At both sites the full PK fertilization caused Ca and Mg concentrations to be lowered (data not shown). In the southern site, Runstorp, the P and K concentrations were low and there were no significant differences between the fertilization treatments. In the northern site, Röbäcksmyran, the full PK fertilization treatment showed higher P and K concentrations in the harvested biomass than the control plots (Figure 1a and 1b). Biomass from the southern site, harvested in November, was all brown, while biomass from the northern site, harvested in October, was still green to some extent. This was probably the reason for the lower K content in Runstorp. Any differences in the K and P content between treatments might have been lost during relocation of nutrients from the shoots to the rhizomes in late autumn in the southern site. Thus we will need to analyse samples from green biomass in the summer to determine if there are any differences in plant uptake of nutrients between the treatments.
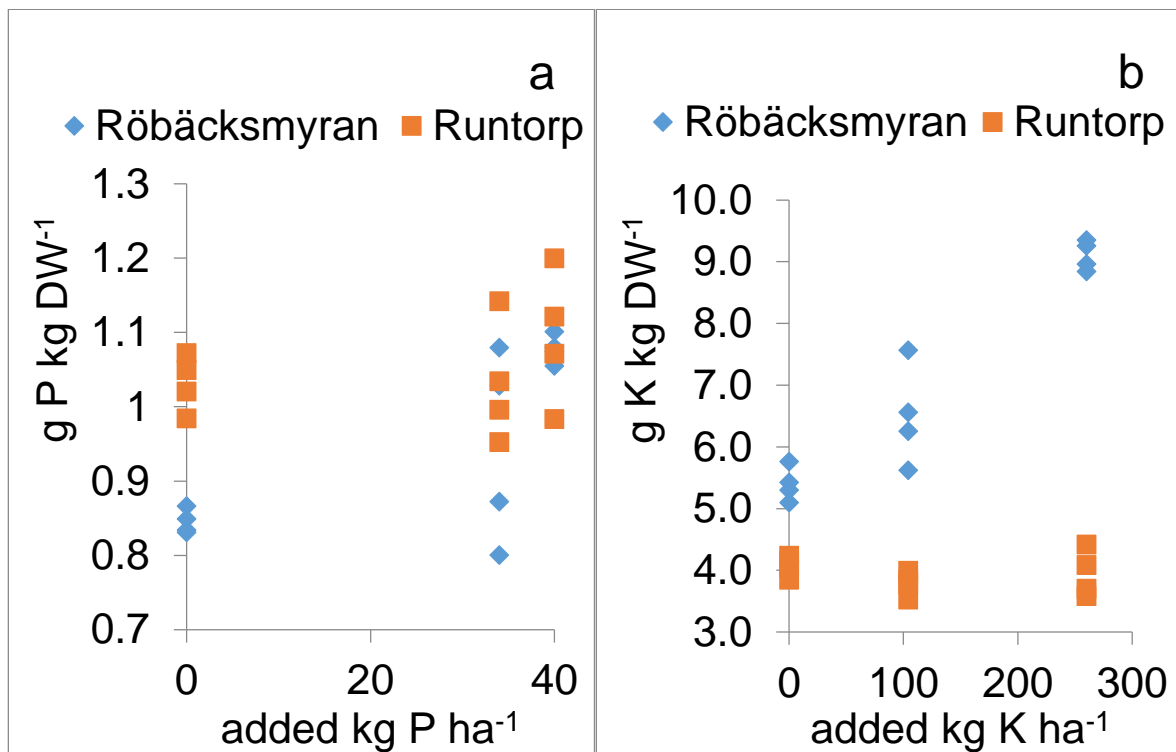
Figure 1. P and K concentrations in harvested RCG biomass in late autumn after 2 experimental years

Plant available nutrients in soil also showed differences between the treatments in the topsoil, but not in the subsoil (Table 1 subsoil not shown). K_{AL} in the topsoil from both sites after full PK fertilization was higher than the unfertilized control and also higher than at the start of the experiment. P_{AL} in both the ash fertilization and the full PK fertilization treatments were higher than the control in the northern site, Röbäcksmyran. It also had increased since the start in the fertilized treatments in Röbäcksmyran and in all treatments in Runtorp.

Table 1. Plant available nutrients (Ammonium lactate extraction) and pH in topsoil (0-20 cm) at experiment start (2012) and after two growing seasons (2013). Means±SE for four replicate samples per block (*= significant $P<0.05$ difference to the control)

Site	Year	Treatment	Ca_{AL}	K_{AL}	Mg_{AL}	P_{AL}	pH
			mg kg* soil^{-1}				
Röbäcksmyran	2012	Before start	998±36	64±4	61±5	28±2	5.4±0.1
	2013	RCG Ash + N	838±51	43±3	46±5	35±2 *	5.5±0.1
	2013	NPK fertilization	833±32	88±12 *	41±2	36±1 *	5.4±0
	2013	N fertilized control	755±52	34±4	35±4	31±1	5.4±0
Runtorp	2012	Before start	1223±47	88±7	46±4	37±2	6.4±0
	2013	RCG Ash + N	1348±91	92±2	44±5 *	51±5	6.3±0.1
	2013	NPK fertilization	1328±90	101±2 *	41±4	50±4	6.2±0
	2013	N fertilized control	1303±86	71±2	37±5	48±5	6.3±0.1

Mg_{AL} after RCG ash fertilization was higher than the control in the southern site, Runtorp. The increases in plant available P and K after full PK fertilization show that the PK demand of delayed-harvest reed canary grass is considerably lower than for forage grasses. Also, the lack of significant yield increases after any of the NPK-fertilized treatments compared to the N-fertilized control, show that the short term demand for P and K in RCG can be satisfied with soil uptake on these loamy soils.

Conclusions

Reed canary grass in a delayed harvest system has a lower demand for P and K than forage grasses. In soils with moderately low P and K availability (Class II in the Swedish system), the short term (two years) demand during the harvest years can be covered by soil supply. However, in the long term, P and K in soil can be depleted and recycling of reed canary grass ash to the fields can be one way to reduce that depletion.

Acknowledgement

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Organic seed production of yellow oat grass – preliminary results

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Abstract

Seed production of mixed crops of yellow oat-grass (*Trisetum flavescens* L. P. Beauv.) and two annual legumes was compared in an organic cropping system with or without using organic manure. The best seed yield was achieved in the cropping system with organic manure, treated by weed harrowing and using black medic as the companion crop (mean of two seed harvest years - 203 kg/ha). This yield is comparable to the yield on conventional control. In a comparison of the two companion legumes, black medic was slightly better than clustered birdsfoot-trefoil. However, the greatest influence on seed yield was from fertilization.

Keywords: *Trisetum flavescens*, seed, organic farming

Introduction

Yellow oat grass (*Trisetum flavescens* L. P. Beauv.) is a valuable forage grass species for organic grassland systems. It is able to produce satisfactory yields of high-quality forage under organic conditions. Yellow oat grass is preferred by animals on pastures, and also gives fine hay with good content of digestible nutrients.

According to the Council Regulation (EEC), seed and vegetative plant material for organic farming should be organic, produced without use of inorganic fertilizers or pesticides. However, sufficient nutrition, mainly of nitrogen, is necessary for achieving favourable seed yield of grasses. In Denmark and Norway, there were attempts to grow grass seed together with legumes (Deleuran and Boelt, 2000; Solberg *et al.*, 2007), predominantly using red and white clovers. However, based on Czech findings (Macháč and Cagaš, 2005) red clover and white clover often suppress the grasses, and so they recommended using black medic as a companion legume. This study was designed to identify which legume species and type of treatment are the best for growing yellow oat-grass for seed on organic farms.

Materials and methods

In 2012-13, a small-plot field trial of organic seed production of yellow oat-grass was conducted at the Grassland Research Station at Zubri. There was no use of any inorganic fertilizers or pesticides on the trial area for the three years before sowing the trial. Yellow oat-grass was undersown in 2010 into spring wheat. In the trial, three experimental factors were combined: (1) companion legume (none, black medic, clustered birdsfoot-trefoil); (2) method of nutrition (conventional control, only by nitrogen using legumes, organic manure); (3) treatment (none, weedy-harrowing – once or twice a year). The sowing rate of yellow oat-grass cv. Roznovsky was 11 kg/ha. Plot size was 10 m^2, with 5 rows with 25 cm row spacing. Legumes were sown across the rows of yellow oat-grass. The sowing rate of black medic (*Medicago lupulina* L.) cv. Ekola was 15 kg/ha and clustered birdsfoot-trefoil (*Lotus ornithopodioides* L.) cv. Junak was 12 kg/ha. On the conventional control inorganic fertilizers were applied (70 kg/ha of nitrogen, 50 kg/ha P+K), and on the plots with organic manure slurry was applied (supplying 70 kg/ha of nitrogen). Applications of both types of fertilization were made in the first half of April. Weed-harrowing was carried out using a plot weeder-harrow (width 1.5 m, work speed 12 km/hod).

In first ley year the mixtures were harvested for forage. Direct combining (plot combine Wintersteiger Elite) of the yellow oat-grass for seed was carried out in the second a third ley years. Harvested seed was cleaned on laboratory screen machines in order to meet purity standards. The data were processed by ANOVA. To evaluate the significance of the differences between means, Tukey test with 5% significance level was used.

Results and discussion

In the first seed-harvest year (2012) the highest seed yield (273 kg/ha) was achieved from the variant with black medic as the companion crop, with organic manure and a single treatment with the weeder-harrow. In contrast, the lowest seed yield (179 kg/ha) was determined at variants without fertilization, with clustered birdsfoot-trefoil as the companion legume and a single treatment by the weeder-harrow. Generally, the seed yield of yellow oat-grass was in very high in the first harvest year and exceeded the average yield in the Czech Republic. In a comparison of experimental factors, statistically significant differences were found for type of treatment, when variants without weed-harrow treatment significantly overyielded the variants treated by the weeder-harrow. Major differences were to be observed for method of fertilization. The highest seed yield was achieved on variants with organic fertilization, which overyielded the conventional control as well as variants without fertilization (Table 1).

Table 1. Effect of trial factors on seed yield (kg/ha) of yellow oat-grass.

Trial Factor	Variant	First seed year			Second seed year		
		Yield	Tukey	rel. (%)	Yield	Tukey	rel. (%)
companion legume	none	225	a	100	142	a	100
	clustered birdsfoot-trefoil	201	a	89	127	b	89
	black medic	217	a	97	128	b	90
	ANOVA	0.404			0.002		
treatment	none	233	a	100	122	c	100
	harrowing 1x	199	b	85	128	bc	105
	harrowing 2x	220	ab	94	134	ab	110
	ANOVA	0.563			0.876		
fertilization	conventional	225	b	100	142	a	100
	only legumes	171	c	76	113	b	79
	organic manure	262	a	116	142	a	100
	ANOVA	<0.001			<0.001		

In second harvest year, the highest seed yield (150 kg/ha) was achieved from the conventional control. In contrast, the lowest yield (94 kg/ha) was observed in variants without harrowing by the weeder-harrow. Significant differences were found for all the trial factors. For type of treatment, all the variants with harrowing overyielded (in contrast to results in previous year) the untreated variants. The highest seed yield was achieved from the variant with double weeder-harrowing. These variants significantly overyielded the variants without harrowing. It is possible to explain that weed-harrowing has a positive effect not only on weed removal of the seed crops, but also on removal of waste material and aeration of the sward, which have more relevance in older swards. In the comparison of types of fertilization, significant differences were found among variants with organic or inorganic fertilization and unmanured variants. The highest yields were observed for the conventional control, organically manured plots gave yields only slightly lower, but variants without fertilization gave yields about 21% lower.

Conclusion

Results of two harvest years brought new findings and information about the possibilities for organic seed production of yellow oat-grass. The unsubstitutable role of nutrition of the grass stand for the achievement satisfactory seed production was confirmed. Organic manure is able to be compensate for conventional fertilization. In the comparison of companion legumes, the

use of black medic was better than clustered birdsfoot-trefoil. Determination of common conclusions will be possible after third, final cycle of field trial in the year 2014.

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Theme 4 'Livestock production'

Theme 4 invited papers

Quality and authenticity of grassland products

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Abstract

Grassland is an important component of the sustainability of ruminant production in many parts of Europe and products from animals on grassland are increasingly valued by the consumer. This paper considers the fatty acid composition, shelf-life and sensory characteristics of meat and milk products of grassland. Animal-based products of grassland have 'added value' among both food producers and consumers because of their perceived healthiness and environmental acceptability. This added-value carries with it an onus to be able to trace and authenticate the food products derived from grassland. A range of techniques has been used to gather data with the potential to discriminate between food products of grassland production and other production systems. Chromatographic and spectroscopic methods, along with mass spectrometry, have been widely used to quantify fatty acids, volatile compounds, carotenoids, tocopherols and stable isotope ratios, and to obtain fingerprint data capable, following multivariate statistical analysis, of discriminating between production systems. Among the challenges to the discrimination process and ultimately to the authentication and traceability of grassland products are the seasonal and geographic variation in the composition of grassland feedstuffs consumed by animals and the difficulty of detecting the consumption of non-grass feedstuffs in a grassland production system.

Keywords: grassland, meat, milk, quality, authentication, traceability

Introduction

Grazed pasture is often a cost-effective feed option for producers. In recent years, pasture-based systems have come to be regarded as more environmentally and animal-welfare friendly alternatives to intensive/feedlot systems of production. There is also growing interest by consumers in the safety, healthiness and quality of their food, its origin, and in the methods by which it is produced. For this review, grassland products are defined primarily as meat and dairy products from fresh pasture. However, while animals may graze predominantly pasture at certain times of the year, e.g. in late spring and in summer, they consume non-grass feeds or conserved forage at other times. Products of conserved forage will therefore be mentioned as relevant to specific issues under discussion. The influence of forage feeding on the fatty acid composition, processing characteristics and sensory quality of milk and meat has been regularly reviewed (e.g. Coulon and Priolo, 2002; Scollan *et al.*, 2005, 2014; Dewhurst *et al.*, 2006). The focus with respect to quality of grassland products will be on recent information on this topic. The Codex Alimentarius Commission defines traceability as 'the ability to follow the movement of a food through specified stages of production, processing and distribution' (WHO/FAO, 2007). Thus, traceability requires a record of the various steps in the journey of a food from its site of production to consumption and 'each link requires keeping records of preceding and succeeding links' (TRACE, 2010). Because traceability systems depend on the maintenance of records, paper or computer based, they are open to error. Authentication, defined as 'the process by which a food is verified as complying with its label description' (Dennis, 1998), is therefore necessary to support traceability systems and to

prove beyond doubt that a particular food product is as is stated on the product label. This paper will focus on the authentication methodologies that underpin such an 'authenticity based traceability system' (TRACE, 2010) for grassland products; i.e. it will focus on authentication of grassland products, and not on traceability *per se*, recognizing that authentication is an essential validation tool for any traceability system.

Quality of meat from grassland

It is recommended that total fat, saturated fatty acids (SFA), omega-6 polyunsaturated fatty acids (PUFA), omega-3 PUFA and trans fatty acids should contribute <15-30%, <10%, <5-8%, <1-2% and <1% of total energy intake in human diets, respectively (WHO, 2003). Meat, fish, fish oils and eggs are important sources of omega-3 PUFA for humans, while beef and other ruminant products such as milk are dietary sources of conjugated linoleic acid (CLA; Scollan *et al*., 2014). The dominant CLA in pasture-fed beef is the cis-9, trans-11 isomer, which has being identified as processing a range of health-promoting biological properties including antitumoral and anticarcinogenic activities (De la Torrre *et al*., 2006). The influence of dietary forage on the fatty acid composition of beef has been recently reviewed (Daley *et al*., 2010; Morgan *et al*., 2012, Shingfield *et al*., 2013, Scollan *et al*., 2014). The findings of the large number of studies now available are generally consistent. Thus, feeding fresh grass compared to concentrates, results in higher concentrations of n-3 PUFA and CLA in muscle lipids. In addition, feeding forage compared to concentrates during the finishing period is frequently associated with a decrease in the concentration of SFA and an increase in the concentration of monounsaturated fatty acids in muscle (Shingfield *et al*., 2013).

With regard to the type of forage, the fatty acid composition of muscle from cattle that grazed alfalfa, pearl millet or a mixed pasture of bluegrass, orchardgrass, tall fescue and white clover before slaughter was largely similar but the concentration of linolenic acid (18:3 n-3) was highest for steers grazing alfalfa (Duckett *et al*., 2013). In contrast, Dierking *et al*. (2010) observed no difference in the fatty acid composition of muscle from steers that grazed tall fescue, tall fescue-red clover rich pasture or alfalfa before slaughter. Similarly, Schmidt *et al*. (2013) observed no difference in the fatty acid composition of muscle from steers that grazed bermuda grass or alfalfa before slaughter, whereas Chiofalo *et al*. (2010) reported a higher 18:3 n-3 in muscle from lambs that grazed subterranean clover compared to those that grazed Italian ryegrass. There is increasing interest in cattle production from botanically diverse pastures but there is a paucity of information on the fatty acid composition of beef produced from such pastures. A general tendency for an increase in n-3 PUFA and total PUFA in intramuscular fat is observed. For a comprehensive review of this topic the reader is referred to Lourenço *et al*. (2008) and Moloney *et al*. (2008).

The bright red colour of beef and lamb is important for consumers when making purchasing decisions. Priolo *et al*. (2001) concluded from their review of 35 studies that muscle from grazing cattle is darker. In contrast, Muir *et al*. (1998) considered 15 publications where beef from cattle fed forage-based diets was compared with beef from cattle fed cereal-based diets but, in which, animals had been finished to comparable slaughter weights or fat-cover levels. They concluded that there were no consistent effects of these feeds on fresh meat colour. Forage feeding leads to beef carcasses with more yellow fat than those from grain-fed animals, through the ingestion, absorption and deposition of carotenoids (mainly β-carotene and lutein).

Since n-3 PUFA are more susceptible to oxidation than n-6 PUFA (Mahecha *et al*., 2010), grass-fed beef should be more susceptible to oxidation which is considered the major cause of meat quality deterioration affecting colour, flavour, and nutritional value (Li and Liu, 2012). However, a consistent finding in the literature is that green forages contribute antioxidants such as α-tocopherol and β-carotene to the meat, which stabilize the fatty acids, increase self-life and

make the meat more desirable when compared with concentrate feeding (Descalzo and Sancho, 2008).

Tenderness is the most important sensory influence on the acceptability of meat, but when tenderness is increased, flavour and juiciness increase in relative importance. When possible confounding influences are removed such as comparing animals at similar weights or fat cover there is little evidence for a consistent difference in tenderness or juiciness between grass-fed and grain-fed beef (Muir *et al.*, 1998).

The flavour of red meats which develops during cooking derives from the Maillard reaction between amino acids and reducing sugars and the thermal degradation of lipid. Diets which alter the fatty acid composition of the lipid fraction of meat could also alter the amount and type of volatiles produced and hence its aroma and flavour. In a comparison of beef from grass- and concentrate-finished animals, the concentrate-fed beef had higher concentrations of linoleic acid (18: 2 n-6) and on cooking produced seven compounds at over three times the level found in the grass-fed beef, which had much higher concentrations of 18:3 n-3 and produced a higher amount of only one compound, 1-phytene, a derivative of chlorophyll ingested with grass (Elmore *et al.*, 2004). Sensory and flavour volatile analysis by Raes *et al.* (2003) showed that grass-fed animals had higher flavour intensity with higher contents of low molecular weight unsaturated aldehydes derived from oxidation of long chain PUFA than grain-fed animals. Vasta and Priolo (2006) reviewed the impact of diet on meat volatiles in ruminants. Animals grazing grass had more of the flavour usually associated with beef meat than the grain-fed cattle (Richardson *et al.*, 2004) whereas in the studies of Tansawat *et al.* (2013), pasture feeding produced more 'barney, greasy and gamey' flavour than grain-fed beef. Scaglia *et al.* (2012) found no difference in flavour in muscle from cattle finished on alfalfa or tall fescue pastures while in the study of Schmidt *et al.* (2013), a greater proportion of consumers preferred beef from cattle that grazed alfalfa compared to beef from cattle that grazed bermudagrass.

Other compounds, not derived from fatty acids, may also contribute to flavour. Skatole has been found in high concentrations in the fat of cattle and sheep that had been fed on grass (Young *et al.*, 2003). Schreurs *et al.* (2008) suggest that the differences in 'pastoral' flavour observed with different forages may reflect their varying propensity to form skatole during rumen fermentation. They suggest a role of condensed tannins-rich plants in ameliorating the development of undesirable flavours in fat of animals consuming white clover or lucerne.

The effect of forage type on flavour appears to be more intensive in lamb and is better documented than for beef. In general, meat from sheep that consumed white clover or lucerne alone has been reported to give a more intense, unacceptable 'sharp' and 'sickly' flavour than meat from grass-fed sheep. In more recent studies, different varieties of legume have been examined in the context of the sensory characteristics of lamb (see Schreurs *et al.* (2008) and Phelan *et al.* (2014).

Increasing the n-3 PUFA intake of beef animals through feeding oilseeds can improve the concentration and proportions of n-3 PUFA in the meat but can also produce an oxidative (and sensory) challenge during retail display. This has been rectified by feeding supplementary α-tocopherol acetate with concentrate diets (see Scollan *et al.*, 2014).

Quality of milk and milk products from grassland

The influence of dietary forage on the fatty acid composition of milk has been reviewed (e.g. Dewhurst *et al.*, 2006; Lourenco *et al.*, 2008). It is well established that the proportions of CLA and n-3 PUFA in milk increase as grass replaces non-grass constituents in the diet of ruminants (Couvreur *et al.*, 2006; Dewhurst *et al.*, 2006, Shingfield *et al.*, 2013). In ewes, Ostrovsky *et al.* (2009) found three times more CLA and twice as much 18:3 n-3 in the milk fat of grazing ewes compared to that of ewes fed a total mixed ration. The CLA concentration decreased two fold when the 18:3 n-3 content in pasture herbage decreased in mid-summer compared to May and

September. Within a grassland production system, milk fat composition varies with stage of maturity of the grass and its botanical composition and between years for similar animals (Butler *et al*., 2011). Several studies have shown elevated levels of 18:3 n-3 in milk and dairy products from cattle grazing Alpine pastures (Dewhurst *et al*., 2006). The Alpine meadows include legumes that are rich in condensed tannins, as well as red clover, which may have reduced rumen biohydrogenation and thereby increased the levels of 18:3 n-3 in milk. From a statistical analysis of available data, Lourenco *et al*. (2008) concluded that milk from cows grazing botanically diverse pasture had increased milk 18:3 n-3 and PUFA concentrations, whereas milk SFA concentrations were in most cases decreased. Kalber *et al*. (2011) reported that berseem clover inclusion in the ration of cows also increases the proportion of 18:3 n-3 in milk, while Larsen *et al*. (2010) found a higher 18:3 n-3 concentration in milk from cows that grazed pastures of perennial ryegrass mixed with white clover, compared to milk from cows that grazed pastures of perennial ryegrass mixed with red clover or lucerne, which did not differ. Cows grazing birdsfoot trefoil (Turner *et al.,* 2005) and sheep grazing Sulla or burr medic (Addis *et al*., 2005) produced milk containing higher levels of 18:3 n-3 than cows grazing non-tanniniferous herbages or sheep grazing other grasses. It appears that this effect is related to reduced biohydrogenation of 18:3 n-3 in the rumen as a consequence of the action of tannins. Petersen *et al*. (2011) noted an increase in 18:3 n-3 when cows were zero grazed a mixture of sown herbs (chicory, plantain, birdsfoot trefoil, white melilot and others) in comparison with zero-grazing white clover-perennial ryegrass. It is not clear which species and mechanisms were involved in this effect.

Dewhurst *et al*. (2006) reviewed the effects of clover silages on fatty acids in milk. In comparison with milk from cows fed grass silages, both red clover and white clover silages led to highly significant increases in the proportion of 18:3 n-3. Van Dorland *et al*. (2008) included red clover silage or white clover silage at 40% of forage dry matter and increased the 18:3 n-3 proportion from 0.9% of milk fatty acids (grass silage control) to 1.04% (red clover silage) and 1.14% (white clover silage). In a meta-analysis of 8 published studies, Steinshamn (2010) statistically confirmed the above findings and reported an average increase in milk 18:3 n-3 proportion from 0.53 to 0.91% due to feeding red clover silage compared to grass clover silage. They found no statistical difference between white clover and red clover silage.

As with meat, the fatty acid composition of milk can also influence the shelf-life and processing characteristics whereby milk with a high PUFA concentration is more susceptible to oxidation and therefore has a shorter shelf-life. Thus, milk from cows fed on red clover silage compared to grass silage contained more 18:2 n-6 and 18:3 n-3 which resulted in increased oxidative deterioration of milk (Al-Mabruk *et al*., 2004). The latter could be corrected by including supplemental vitamin E, an anti-oxidant, in the rations of the cows. Similarly, Havemose *et al.* (2006) reported a lower concentration of lipid hydroperoxides in milk from cows fed hay compared to those fed grass-clover silage, which reflected the higher 18: 3 n-3 concentration in the latter milk. In contrast, Adler *et al*. (2013) observed a lower concentration of hydroperoxides in milk from cows that grazed a grass-red clover pasture compared to a grass-white clover pasture despite no difference in 18:3 n-3 concentration. The authors suggested that this reflected differences in the concentration of vitamin E in the milk.

The sensory quality of dairy products can be influenced by animal diet (reviewed by Coulon *et al*., 2004; Martin *et al*., 2005a). The fatty acid composition of milk may play a role in flavour; e.g., oxidized milk and milk products are characterized by metallic, cardboard or stale flavours and production of oxidized flavour at 8 days post-sampling was positively correlated with levels of 18:2n-6, 18:3n-3 and total PUFA in milk fat (Timmons *et al*., 2001). Croissant *et al*. (2007) reported that milk from cows fed a conventional ration was characterized by 'a sweet feed/malty flavour, a greater sweet aromatic flavor, and a sweet taste, but no grassy or mothball flavours' compared to pasture-based milk. Moorby *et al*. (2009) reported that milk from cows fed red

clover silage was rated 'more boiled' and was whiter and thinner textured compared to milk from cows fed grass silage. Larsen *et al*. (2013) reported that replacement of maize silage with lucerne silage resulted in milk that was more yellow and had 'less creamy flavour and less stale aroma'. Couvreur *et al*. (2006) observed that rancidity of butter (flavour and odour) decreased when the proportion of fresh grass in the diet increased, which the authors ascribed to a decrease in the proportion of 14:0 in milk fat.

The relationship between the nature of dietary forage and the sensory characteristics of milk and milk products has been examined in France in the context of dairy products with a protected designation of origin (PDO) and their 'terroir'. Martin *et al*. (2005a) in their review indicate that differences in the sensory characteristics of cheese made from milk produced in the valleys or in the mountains are related in part to the presence of legumes in the pastures grazed by the cows (see also Moloney *et al*., 2008). Claps *et al*. (2009) reported that cheese made from goats that grazed *Medicago sativa* had less intense taste but was similar in acceptability to cheese made from milk from goats that grazed *Lolium perenne*.

As with meat, other dietary compounds may also contribute to flavour. Terpenes have aromatic properties and abound in certain aromatic dicotyledons species found in diversified meadows (Mariaca *et al*., 1997). These molecules are found in higher concentrations in cheese when the animals are fed dicotyledon-rich natural grass forage (Viallon *et al*., 2000). However, it appears that the increase in terpene concentration in cheese is generally not sufficiently large to exert any marked effect on flavour (Bugaud *et al*., 2001).

Food authentication techniques

Strategies to authenticate grassland products have focussed firstly on the measurement of components that directly reflect the diets consumed (see above). Secondly, a 'fingerprint' approach can be taken whereby spectroscopic techniques are used to determine differences in the optical properties of foods derived from different production systems. Transcriptomic and proteomic techniques are now being also being evaluated in this regard (Hocquette *et al*., 2009; Shibata *et al*., 2009). Several recent reviews describe the methodologies now available for application to authentication of meat and milk (e.g Karoui and Baerdemaeker, 2007; Luykx and Van Ruth, 2008; Sun, 2008; Monahan *et al*., 2010, Capuano *et al*., 2013).

Fatty acid data

In a recent study (Roehrle, 2014), a discrimination model of muscle fatty acid data permitted differentiation of beef from animals raised on grass, a barley-based concentrate or on grass-concentrate combinations over a 12-month period with a correct classification of 92.9%. The mis-classified samples related to beef from animals raised on pasture for 12 months prior to slaughter being classified as 'beef from animals fed grass silage for 6 months followed by grass at pasture for 6 months.' Effectively, however, both groups could be considered grass-fed, since the silage is ensiled grass; therefore the mis-classification is not of major significance from the perspective of grass-fed beef authentication, and pooling these groups together gave 100%-correct classification of beef according to diet. A similar approach has been used to distinguish organic milk from conventional milk (Molkentin and Giesemann, 2007) and upland from lowland milk (Engel *et al*., 2007). Povola *et al*. (2012) using a similar approach showed promising separation of cheese made from milk of cows that grazed *Trifolium alpinum* or *Festuca nigrescens*.

Vetter and Schroeder (2010) attributed higher levels of phytanic acid, and its degradation product pristanic acid, in organic dairy products compared to conventionally-produced dairy products, to the predominant use of grass-based feedstuffs in organic production. These authors set a target value of at least 200 mg phytanic acid/100 g lipid for the verification of grass-fed, organic dairy products. However, this assumes that all conventional production is 'less' grass-based and uses diets that are sufficiently different from the organic diets.

A potential limitation to the use of fatty acid profile as an indicator of grassland production is that non-grass sources of fatty acids could give 18:3 n-3, 18:2 n-6 or CLA contents in meat and milk similar to those derived from grass (Shingfield *et al*., 2013).

Volatile compounds

Among the meat volatile components influenced by diet are branched chain fatty acids, lactones, aldehydes, phenolic compounds, indoles, 2,3-octanedione, terpenes and sulphur compounds (Vasta and Priolo, 2006). Some compounds are directly incorporated into tissues from the dietary components while others are generated during cooking of meat fat (see above). In a study of beef from animals raised at pasture, on concentrates or on grass silage-pasture-concentrate combinations, skatole, 3-undecanone, cuminic alcohol and 2 methyl-1-butanol were identified as compounds that allowed discrimination between beef from animals animals fed pasture or concentrates or combinations thereof (Vasta *et al*., 2011). Germacrene D, a terpenoid, was a marker of grass feeding.

Priolo *et al*. (2004) identified four terpenes in ovine fat, from a total of 33 terpenes detected, which permitted discrimination of lamb from sheep raised and finished on pasture from that of sheep raised on concentrate or concentrate-pasture combinations. Serrano *et al*. (2011) reported complete separation of suckling bulls supplemented with hay, cut green herbage or grazed pasture by measuring 2,3-octanedione, skatole and terpenes in adipose tissue. The terpenoid content and profile of pasture herbage depends on plant species, stage of growth and grazing management (Mariaca *et al*., 1997; Tornambé *et al*., 2006) so it is therefore not easy to conclude that terpenes generally, or indeed specific terpenes, are higher or lower in one production system compared to another. In milk and cheese, Martin *et al*. (2005b) reviewed a number of studies on the discrimination of milk and cheese from cows fed different diets. Analysis of terpenes was used to discriminate between milk from two regions of France in both summer and winter with geographical discrimination attributed to botanical differences in the forages. Terpene transfer from forages to milk was shown to be fast, as early as the first milking after consumption, and terpenes were transferred into cheese with minor alteration.

Stable isotopes analysis

Camin *et al*. (2007) demonstrated the potential for C, N, H and S analysis to discriminate between lamb sourced in different parts of Europe. The influence of grassland production was clearly evident, with lamb samples from island sources on the western seaboard of Europe (Ireland and the Orkney islands) clustering separately, mainly on the basis of lower $^{13}C/^{12}C$ ratios, from samples originating in mainland Europe, where supplemental cereal or maize-based inputs may be fed for periods of the year. Bahar *et al*. (2008) found differences in the stable isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$) of organic and conventional beef sourced from retail outlets. The extent of differences was seasonal with pronounced differences in stable isotope ratios between the two beef sources in the months after winter feeding and similar stable isotope ratios after summer grazing. However, recent studies have shown that it is possible to discriminate between meat from animals fed different C_3 plant sources despite the relatively close $^{13}C/^{12}C$ ratios of the diets (e.g. Osorio *et al*., 2011)

Rossmann *et al*. (2000) highlighted the potential of the stable isotope technique (C, N, O and S) when combined with measurements of other markers including fatty acids, carotenoids and trace elements for the discrimination of butter from different regions. In cow's milk, Renou *et al*. (2004) demonstrated differences in $^{18}O/^{16}O$ between milk of animals raised in two regions of France (Brittany vs. the Massif Central). They also showed differences between milk in the Massif Central produced in spring from pasture, in winter from grass silage and in winter from hay, but in Brittany there was no difference between milk produced in winter from maize silage and in winter from hay. While differences between sites may reflect differences in the drinking water $^{18}O/^{16}O$ the contribution of water from feedstuffs must be considered, which can either

eliminate or accentuate inter-site differences. Bontempo *et al.* (2012) concluded that stable isotope ratios of H, C, N and O of milk and cheese are linked to 'the terroir, in particular to the type of vegetation and the environment'.

Optical properties and carotenoids

Prache and co-workers have examined the application of reflectance spectroscopy in the visible region (450-510 nm) to discriminate between lamb production systems (reviewed by Prache, 2009). They advocate measurement of carotenoids in both adipose tissue and blood to lower the likelihood of mis-classification. Issues that may limit the usefulness of this approach include the relatively slow rate of change of carotenoids in tissue following a change in diet and the variation in carotenoid concentration in pasture. In addition, since depletion of carotenoids in adipose tissue, after a change to a low carotenoid diet, occurs due to a dilution of existing adipose tissue by new adipose tissue; in mature animals carotenoid measurement in adipose tissue may not be an appropriate indicator of diet.

In agreement with the work of Prache and co-workers, (Röhrle *et al.*, 2010a) showed contrasting reflectance spectra (400-700 nm) for subcutaneous adipose tissue from animals fed pasture (P) vs. a barley-based concentrate (C) for a 12-month period. Furthermore, subcutaneous adipose tissue from a group fed silage for 6 months followed by pasture for 6 months (SiP) was distinguishable at slaughter from that of the group that fed on pasture for 12 months, indicating an effect of a diet consumed 6 months earlier on adipose tissue reflectance at slaughter. However, a group fed silage for 6 months followed by a 50:50 (DM basis) pasture-concentrate mixture for 6 months (SiPC) was not distinguishable from the SiP group, undermining somewhat this methodology as a means of diet discrimination.

Noziere *et al.* (2006) demonstrated the complexities associated with attempting to relate carotenoid content of milk to production system. While recognizing that the carotenoid content of fresh grasses is higher than that of conserved forage and concentrates, seasonal variation in fresh grass carotenoids affected by the stage of growth, is also a contributory factor. Practical factors such as the pooling of milk and its bulk storage prior to processing pose a significant challenge in terms of authentication of milk or the processed dairy products derived from it. Reflectance measurements have also been applied to milk in an attempt to distinguish between grass vs. hay and concentrate feeding (in individual cows) – this was possible provided there was a least a 36-day interval between time of diet switch from the low carotenoid (concentrate, hay) to the high concentrate (pasture) diet (Noziere *et al.*, 2006).

Near and mid infra-red spectroscopy (NIRS and MIRS, respectively) has also shown promise as a tool for authenticating the products of grassland. Dian *et al.* (2008) examined the ability of spectroscopy between 400 and 2500 nm and reported that the longer wavelength range produced models of higher discriminant ability producing correct classification rates of 97.7%. Valenti *et al.* (2013) concluded that MIRS was better than NIRS when discriminating between milk from cows offered hay or pasture-based diets. Gori *et al.* (2012) further showed that Fourier transform infra-red spectroscopy coupled with artificial neural networks could distinguish the dietary regime of butters produced in the Parmigiano Reggiano cheese region in Italy.

Vitamin E stereoisomers

Analysis of stereoisomeric forms of α-tocopherol in animal tissues can give information about whether animals received vitamin E from natural or synthetic sources. In muscle from grass-fed beef cattle the RRR stereoisomer dominated, while in concentrate-fed animals and in beef of unknown dietary background stereoisomers of synthetic origin were evident (Röhrle *et al.*, 2010b).

Functional genomics

Cassar-Malek *et al.* (2009), in a comparison of outdoor pasture vs. indoor concentrate feeding of Charolais cattle, found Selenoprotein W to be under-expressed in pasture-fed animals and proposed it as a putative gene marker of the grassland system. Duckett *et al.* (2009) studied expression of genes involved in lipogenesis in muscle and found up-regulation of stearoyl-CoA desaturase, fatty acid synthase and Spot-14 and down regulation of signal transducer and activator of transcription-5 (STAT5) in the subcutaneous fat of grazing steers finished on a high-concentrate diet compared with a pasture only diet. Shibata *et al.* (2009) showed that differential expression of muscle proteins occurred during the fattening period in concentrate-fed vs grazed cattle.

To obtain useful information from the data collected by fingerprint methods or where multiple variables are measured, multivariate statistical procedures, often termed chemometrics, are required, e.g. Karoui and De Baerdemaeker (2007) for dairy products. Chemometric methods may be applied to complete datasets or after a variable reduction procedure has been applied; in the case of spectral data in particular, the raw data may also be pre-treated mathematically to reduce or remove interferences caused by physical factors related to the sample.

Conclusions

Modern consumers have a greater interest in the environment, animal welfare and the origin and method of production of their food than heretofore. This is reflected in growing preference for food products of pasture-based systems of production. A substantial body of information is now available on the differences in composition and sensory properties of products from pasture-based and concentrate based systems of production. For meat, current research seems to be focussed on the relative effects of pasture species and varieties. For milk, there appears to be less focus on grassland in this regard, presumably because, in general, grassland represents a smaller proportion of diet of the lactating compared to the growing animal. Emerging data indicate that milk and meat produced from botanically diverse pastures have higher concentrations of fatty acids and anti-oxidants which are considered to be of benefit to human health.

Much progress has been made in recent years in advancing our capabilities in the area of food authentication. Challenges clearly remain in applying authentication methodologies to products of grassland because potential markers are influenced by the complexities of pre-slaughter diets available to animals and of the production systems themselves. Novel approaches are required to overcome these hurdles. These are likely to involve, initially, the use of multiple methodologies to measure multiple markers in multiple tissues and advanced chemometric techniques. Rather than developing authentication methods for broad categories of animal-derived foods, such as 'grass-fed', 'organic', 'free range' or 'extensive' production, a more effective approach may be to develop authentication methodologies for products produced locally and to specific restricted feeding regimes. Animal products produced to a particular feed 'recipe' in a specific location are more likely to hold a unique marker fingerprint to differentiate them from other products and thus underpin an authenticity-based traceability system associated with their production. From a grassland perspective, simplification of the production process so that high-value products are produced to a recipe which can be easily validated by authentication methodologies, but which cannot be mimicked by fraudulent production practices, may be the most promising way forward.

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Sustainable intensification of grass-based ruminant production

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Abstract

The sustainable or ecological intensification of grass-based food production systems provides an opportunity to align the ever increasing global demand for food with the necessity to re-green ruminant production. The challenge for production scientists now is to find innovative ways to improve grass-based production processes to maximize resource use-efficiency based on improved management practices. The objective of this paper is, firstly, to outline the potential opportunities to enhance the yield and quality of grasslands for grazing and conserved forages paying particular attention to species diversity and legumes. Subsequently, the paper addresses the necessity to choose appropriate animals and management practices to improve productive and reproductive performance within such systems. Finally, the paper reports experimental results from dairy cow and sheep production systems that succeeded in combining high animal performance with low environmental impacts.

Keywords: grass yield, quality, legumes, animal productivity, grazing, dairy cow, sheep

Introduction

Global food demand, climate change, urbanization and bio-fuel production will increase competition for agricultural land use between crop and herbivore production. Consequently, it is expected that ruminant production will have to be concentrated on non-arable lands (permanent grasslands) and on arable lands where it would be the most profitable system of production.

European grass-based ruminant production systems face a threefold challenge: i) to meet the rapidly changing demand for food within a resource-constrained environment; ii) to do so in an environmentally and socially sustainable manner for consumers and producers; and iii) to ensure that the products produced meet the highest standards of quality and nutritional value for increasingly discerning consumers. Producing more food from the same land area, while reducing environmental impacts, requires what has been referred to as 'sustainable intensification' (Pretty, 1997) or 'ecological intensification' (Griffon, 2013) of agricultural production. This is based on new innovative blueprints of production based on increased herbage production and quality, and improved utilization under grazing.

The aim of the present contribution is to propose a framework of sustainable intensification in grass-based ruminant production. The definition and challenges of sustainable intensification in grass-based ruminant production will first be outlined. Then specific aspects, including grass production and quality, the type of animals and management practices will be addressed.

Sustainable intensification: definition and challenges

Since the 1970s, and the growing environmental concern over industrial agriculture and livestock production, there has been a consensus in society and the scientific community on the necessity to re-green agriculture. However, the best approach to achieve re-greening is still a matter for much debate among production scientists, legislators and other public stakeholders, and numerous concepts can be found in the literature (Griffon, 2013): 'sustainable agriculture', 'conservation agriculture', 'agro-ecology', 'organic farming', 'high nature-value agriculture', 'ecological or sustainable intensified agriculture' *etc*.

Ecological intensification of agricultural systems was recently defined by Hochman *et al.* (2013) as producing more food per unit of resource used, while minimizing the impact on the environment. Griffon (2013) stated that ecological intensification contrasts with chemical and energy use for crop production and with the use of feed and drugs for animal production. Thus, ecological intensification relies more heavily on the use of natural resources and the functionality (or ecosystem services) they provide and it places less emphasis on the use of external inputs. The challenge for such systems is to improve the efficiency of natural resource use in order to increase food production from existing farmland while minimizing pressure on the environment.

The literature on ecological intensification and agro-ecology in animal production is scarce. Recently, Dumont *et al.* (2013) proposed the development of ecology-based alternatives for animal production by adopting management practices to improve animal health, decreasing the inputs needed for production, decreasing pollution by optimizing the metabolic functioning of farming systems and by adapting management practices to enhance biological diversity and strengthen the resilience within animal production systems.

Grass-based systems have been shown to be beneficial to the environment (Jankowska-Huflejt, 2006; Peyraud *et al.*, 2010) and to be economically successful by reducing total costs of food production (Dillon *et al.*, 2005). However, systems based on increased mobilization of services provided by natural resources (low supplementary feed input or organic systems) have to cope with more variability in relation to plants, animals, climate and bio-aggressors. Such systems therefore require plants and animals that are robust and easy to manage. The production performance of grassland is dependent on the yield of utilizable energy and protein in the grass grown. This means that increasing the yield and stability of high quality grass growth is imperative to ensure the robustness of the overall system. The animal required for efficient grassland-based production systems must be robust, autonomous and 'easy care', and capable of high levels of performance from a predominantly grazed pasture diet. Finally, the management of such systems has to maximize the utilization of a **renewable low cost resource without adverse effects on the environment over the long term.**

Achieving and maintaining optimum soil fertility is a prerequisite for high productivity grassland production systems. The topic of soil fertility is, however, outside the direct scope of this paper. In order to maximize nutrient-use efficiency within grass-based production systems, and to minimize environmental impacts, nutrient recycling must be improved by closely matching nutrient supply to grassland demand according to the climatic conditions.

Combining yield and quality of grasslands for grazing and conserved forages

Forage production and forage quality must be increased without increasing the negative effects on the environment. In recent years research efforts have moved from improving the production and quality of single-species swards through breeding and fertilization to focus on the role of diversity and thus multispecies sown and permanent swards.

The role of species diversity in sown and permanent swards

In a recent review of the literature, Huyghe *et al.* (2012) showed that a positive relationship between species diversity in sown swards and biomass production is frequently found in controlled environments. More so than the number of species, the functional diversity (type of grasses, legumes, forbs) has a positive effect on forage yield (Kirwan *et al.*, 2007), and the highest yields are often achieved with intermediate species diversity.

The relationship between species diversity and herbage feed value has received little attention. In an experiment reported by Huyghe *et al.* (2008) in which species diversity ranged from one to eight species, the negative relationship between forage yield and *in vitro* digestibility was not affected by the number of species nor by the number of functional groups. A notable advantage

of complex swards combining grasses and legumes was the more stable chemical composition across the year that allows more flexibility in sward utilization for grazing and harvesting.

The functional composition is also a key feature which explains variation in productivity and quality and their temporal patterns in permanent grasslands (Duru *et al.*, 2010). In a recent study in France on a set of 190 permanent grasslands representative of most pedo-climatic conditions from Atlantic to Alpine areas (Michaud *et al.*, 2014), feed value, both in early and late spring, was positively related to the proportion of legume species in the sward. A higher stability of forage quality in spring was related to high proportions of forbs and conservative grasses (Figure 1).
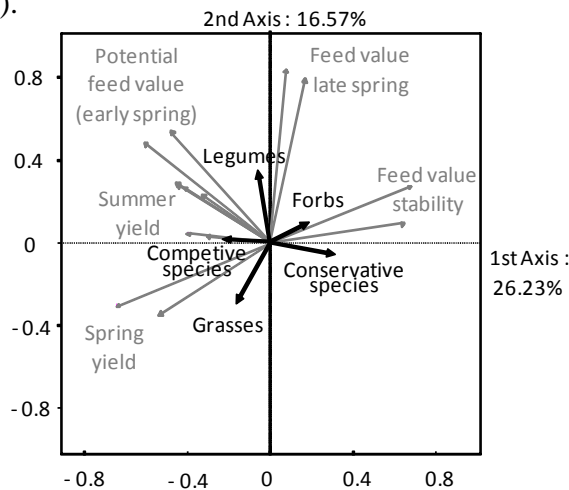
Figure 1. Principal component analysis of herbage yield, feed value and functional composition on a set of 190 permanent grasslands in France (from the data of Michaud *et al.*, 2014)

Species diversity may have a positive effect on voluntary food intake, as diversity offers a choice of herbage species and previous studies have reported that choice significantly increases intake at pasture in sheep (Cortes *et al.*, 2006) and indoors with conserved forages (Ginane *et al.,* 2002 with heifers, Bruinenberg *et al.*, 2003 with cows). It is not clear whether the variation in intake is a consequence of the diversity *per se* or of the choice situation, even though the experiments of Cortes *et al.* (2006) support the hypothesis of a true effect of diversity. In contrast, Soder *et al.* (2006) found no effect of species diversity on dairy cow intake, suggesting that the animal type could interact with the species diversity effect.

The specific role of legume species

Introducing legume species into conserved and grazed forages gives many advantages relating to feed value, animal performance and environmental impact.

White clover has a high digestibility and a high energy value; this is attributed to its low fibre concentration which reflects the absence of structural components such as stems and sheaths (Ayres *et al.*, 1998). A particular advantage of white clover is the reduced rate of decline in digestibility in the mid-season compared to perennial ryegrass (Ulyatt, 1970). Furthermore, white clover supports increased voluntary intake (Ribeiro Filho *et al.*, 2003). Increased production performance as a result of increasing the sward white clover proportion has been observed in dairy cows (Dewhurst *et al.*, 2003), beef steers (Thomas *et al.*, 1981) and sheep (Orr *et al.*, 1990). The results depend on the proportion of white clover in the sward. Egan *et al.* (2013) found that cows grazing a grass-clover sward (21.6% clover content) had higher milk yield and milk solids yield than cows grazing a grass-only sward, whereas Enriquez-Hidalgo *et al.* (2012) found no difference in milk yield and milk solids yield when the average sward clover content was only 13%.

When expressed on a proportion of gross energy intake or unit-intake basis, methane emissions are often lower for forage legume-fed animals than grass-fed animals (McCaughey *et al.*, 1999; Waghorn *et al.*, 2002). Beauchemin *et al.* (2008) proposed that this was due to the lower fibre content, higher dry matter intake, increased passage rate and presence of condensed tannins

(CTs) in certain legume species such as *Lotus corniculatus* or *Onobrychis viciifolia*. Although the effect of CTs on methane emissions has been found in several studies *in vitro* (e.g. Niderkorn *et al.*, 2011), it remains uncertain *in vivo*.

Condensed tannins are also important in reducing the degradation of forage proteins in the rumen, without reducing the amount of microbial protein which is synthesized, and in deactivating internal parasites (Min *et al.*, 2003). These features have positive implications for nitrogen excretion and the use of anthelmintic drugs respectively. Promising results were obtained with *Onobrychisv viciifolia*, with similar voluntary intake and digestibility as lucerne, but lower protein degradation in the rumen resulting in lower protein excretion in urine (Theodoridou *et al.*, 2010). Another interesting secondary compound is polyphenol oxidase, which is present in red clover and which was shown to inhibit proteolysis and lipolysis (Lee *et al.*, 2004). These properties could improve silage conservation, as CTs also do (Theodoridou *et al.*, 2012), and limit fatty acid hydrogenation in the rumen. A challenge for the future is to find positive associative effects between plants, relying on synergies between the different bioactive compounds (Niderkorn and Baumont, 2009; Aufrère *et al.*, 2012): e.g., could CTs from one plant bind with proteins from another plant? Recently, synergies between cocksfoot silage and red clover silage, and between ryegrass and chicory, were observed for DM and NDF intake and eating rate, with 50:50 being the optimal proportions. For the cocksfoot silage-red clover silage association, the synergistic effect was also observed on daily digestible organic matter intake (Niderkorn *et al.*, 2014).

Quality vs. quantity: biomass accumulation and stage of maturity

As biomass is accumulating with plant growth, sward quality in terms of net energy, protein content and potential voluntary intake is decreasing as a result of plant maturation. This is well documented for single-species swards of grasses and legumes, e.g. in the feed value tables used in France (Baumont *et al.*, 2007). A less mature plant contains a lower proportion of true stem and dead material and a greater proportion of leaf which is lower in fibre and highly digestible (Curran *et al.*, 2010; Beecher *et al.*, 2013). As the plant enters the reproductive stage, leaf proportion decreases and stem proportion increases, with negative effects on sward digestibility, crude protein concentration and voluntary intake.

Thus, harvesting and grazing management have to deal with the trade-off between forage quantity and quality. In spring, for conservation, the forage should be cut at the beginning of grass heading to maximize net energy and protein harvested per ha. The decrease in feed value between the beginning and end of heading will necessitate 2.5 kg/day more concentrate for a dairy cow to produce 30 kg of milk. At grazing, increased frequency of defoliation results in high quality but a decrease in net herbage accumulation whereas infrequent defoliation leads to greater herbage production, but decreased grass feed value (Hoogendorn *et al.*, 1992; Beecher *et al.*, submitted). High biomass yield (kg DM ha^{-1}) at grazing will limit animal performance through digestive constraints (low intake of poorly digestible matter), but high quality swards (low biomass yield) can also limit animal performance through behavioural constraints if the time required to graze the required quantity of grass is too great (Baumont *et al.*, 2004). The effect of biomass yield at grazing may also vary over the grazing season. Tunon (2013) found no effect of biomass yield up to 2,300 kg DM ha^{-1} (>4 cm) from April to July, but observed a reduction in milk fat plus protein yield compared to 1,000 and 1,500 kg DM ha^{-1} swards from July to October. McEvoy *et al.* (2010) found similar results. This agrees with work by Beecher *et al.* (submitted) who observed no difference in OM digestibility between swards with a biomass <1500 kg DM ha^{-1} and >2000 kg DM ha^{-1} in spring. In summer and autumn however, increasing biomass yield resulted in a significant decrease in OM digestibility and in digestible OM intake (Figure 2).
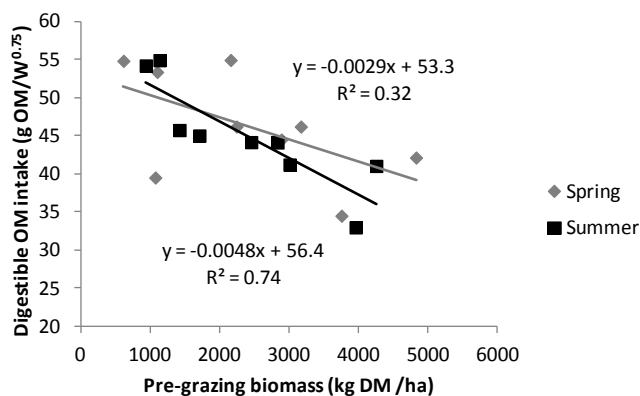
Figure 2. Effect of pre-grazing biomass on digestible dry matter intake measured in sheep (data from Beecher *et al.*, submitted)

Increasing grass quality via the use of less-mature grass also has positive implications for product quality (Hoogendorn *et al.*, 1992) and environmental measures. For example, biomass yield had no effect on enteric methane emissions in spring, but in summer, grazing swards of high biomass resulted in higher emissions (Wims *et al.*, 2010).

Finally, to manage the trade-off between quantity and quality, grazing consistently very low biomass yield swards (<1,200 kg DM ha^{-1}) should be avoided during the main grazing season as this can depress pasture regrowth rate. Diversified swards in which species have different growth and maturation rates and the use of late-heading cultivars could help manage the trade-off between quantity and quality by smoothing the biomass accumulation and the associated decline in feed value.

Choosing the appropriate animal for sustainable grass-based production

As only ~10% of the world's milk production comes from grazing systems, the majority of global ruminant livestock have not been selected for grazing systems. The long running scientific debate on the importance of genotype × environment interactions has been refuelled in recent years as the interest in grass-based systems in Europe has increased. Until recently, most experimental results have indicated little or no importance of such interactions (Holmes, 1995); however, increasingly diverse genotypes and/or production environments have increased the likelihood of such interactions (Falconer, 1990). There is now strong evidence to show that the animals that are genetically best suited to non-grazing systems, are not suited to grazing systems (Delaby *et al.*, 2010).

Animals for grass-based systems

Successful grazing systems require animals capable of achieving large intakes of forage relative to their genetic potential for production so that they can achieve their nutritional requirements almost entirely from grazing, with some conserved forage. As animal intakes at grazing are reduced relative to confinement systems, efficient animals within grazing systems have moderated feed requirements which are consistent with the feed supply capability of grazing. Consequently, such autonomous animals at grazing can achieve high milk production (and composition), retain optimal reproductive capacity and maintain adequate body reserves to avoid ill health within a restricted feed environment (Delaby *et al.*, 2009; Cutullic *et al.*, 2011). Additionally in the context of sustainable intensification, the necessity for more animals to be managed by individual farmers requires more robust autonomous livestock requiring less individual managerial assistance. Animals for grazing systems must also be able to graze effectively and to walk long distances, abilities that are not required in confinement systems.

The use of alternative breeds or crossbreeding to satisfy the specificities of an animal suited to the grass-based system is now being considered by farmers in many countries. Dual purpose or cross breed cows seem more flexible and better adapted to grazing and have improved health,

milk value, reproductive performance, feed efficiency and beef value. Recent studies have examined the suitability of alternative cow breeds for grass-based milk production systems in both France (Delaby *et al.*, 2014) and Ireland (Coleman *et al.*, 2009; Prendiville *et al.*, 2009). In France, an experiment run at the INRA experimental farm Le Pin-au-Haras (Normandy) evaluated the ability of different types of dairy cow to produce and to reproduce in response to two contrasting feeding strategies in a compact calving context (Table 1).

Table 1. Milk and reproductive performance of dairy cows according to breed and feeding strategy (from the INRA experiment in Le Pin-au-Haras; n=380 lactations)

Breed	Holstein		Normande	
Feeding strategy	High [1]	Low [2]	High [1]	Low [2]
Milk yield (44 weeks - kg)	8515	6022	6332	4798
Fat content (g/kg)	38.0	39.5	41.0	41.8
Protein content (g/kg)	32.1	31.0	34.9	33.3
Milk Solids (kg)	587	418	466	351
Body Condition Score change (pts)	-1.00	-1.25	-0.60	-0.90
First insemination success (%)	28	20	41	38
Recalving (%)	59	44	71	68

(1) The High feeding strategy had maize silage, grass silage, dehydrated alfalfa and concentrates in the indoor diet, a higher stocking rate, and supplementation with maize silage, grass silage and concentrate during the grazing season.
(2) The Low feeding strategy had grass silage and haylage in the indoor diet, a lower stocking rate, and supplementation with grass silage during the grazing season.

The high reactivity of the milk production in Holstein cows, as well as high body condition score loss and poor reproduction performance, makes the Holstein cow incompatible with the herbage system with no concentrate input and compact spring calving. In contrast, the Normande, a dual-purpose breed, appears less sensitive and better adapted to low input systems based on the maximization of grassland use for milk production. In Ireland, Prendiville *et al.* (2009) compared the biological efficiency of three genotypes (Jersey, Holstein-Friesian and Jersey × Holstein-Friesian) within grass-based systems. They reported higher milk production efficiencies among Jersey × Holstein-Friesian cattle compared to purebred Holstein-Friesian cattle. In comparison with purebred Holstein-Friesian dairy cattle, Jersey × Holstein-Friesian cattle achieved improved reproductive performance, a greater intake per kg of body weight at grazing and consequently, required 12% less grass to produce 1 kg of milk fat plus protein. Differences in intake and varying measures of feed conversion efficiency between dairy cows of various pure breeds have also been reported previously (for review, see Grainger and Goddard, 2004).

Animal management for grass-based systems

Grass-based systems of milk production require compact calving in spring to match feed supply and herd demand. This is based on achieving high rates of pregnancy within a short period of time following the start of breeding. Calving date is an important determinant of milk production and feed utilization in grass-based systems, through its impact on the alignment of feed demand with supply. Altering the mean calving date of the herd may have a role in reducing the reliance of grass-based farm systems on purchased feeds particularly at higher stocking rates. Both Dillon *et al.* (1995) and McCarthy *et al.* (2013) observed that delaying calving until March achieved a better alignment of dairy herd requirements and grass growth within Irish grass-based milk production studies, increased milk production from grazed grass, reduced the

requirement for purchased supplements and achieved a greater efficiency of energy utilization particularly at higher stocking rates.

In sheep production, high ewe productivity (number of lambs produced per ewe per year × mean lamb carcass weight) is a key indicator to ensure economic sustainability. In organic sheep production systems, where hormonal treatments are prohibited and the price of concentrate feed increases the pressure on production costs, increasing reproductive capacity could be used to increase ewe productivity without undue penalty for other technical or economic performance targets. Increasing ewe productivity by increasing ewe reproductive capacity was evaluated on a rustic breed (Limousine) over a four-year period. In the study, one lambing per ewe per year (*1/year*) (with 50% lambings in spring and 50% in autumn) was compared to three lambings over two years (*3in2*) (Benoit *et al.*, 2009). Ewe productivity in *1/year* (1.51) was slightly lower than in *3in2* (1.61), but with a lower between-year variability, lower lamb mortality and parasitism level and lower concentrate feed consumption per ewe. Lamb carcass conformation, fatness and fat colour were not different between systems, but carcass weight and subcutaneous dorsal fat firmness were lower in *3in2* lambs than in *1/year*. Intensification in an organic sheep system through increased reproduction rhythm therefore did not lead to better animal performance nor economic results and proved riskier, more variable and more difficult to manage, and thus less sustainable. The less intensive system (*1/year*) was highly efficient from the animal perspective and highly food self-sufficient (Benoit *et al.*, 2009).

Improving the efficiency of resource use in grazing systems

Grazed grass is the cheapest feed source (Finneran *et al.*, 2012) and commonly comprises 0.60 to 0.90 of ruminant animal diets within grass-based systems in Europe. Therefore, the production and utilization of increased quantities of higher quality grazed grass, coupled with the close alignment of grass production and animal requirements, has the potential to increase overall system productivity and contribute significantly to the sustainable intensification of agricultural production. Further research is therefore needed to develop management practices and technologies that will facilitate increases in milk and meat output from sustainable grass-based systems.

High stocking rates in grass-based dairy systems can be compatible with high environmental performance

Stocking rate (SR), traditionally defined as the number of animals per unit area of land used during a defined period of time, is widely acknowledged as the main driver of productivity from grazing systems and this applies across all grazing species (Rattray, 1987; Hoden *et al.,* 1991; Baudracco *et al.,* 2010; Crosson and McGee; 2011) due to its dominant effect on animal demand and hence pasture use. Increasing SR is usually associated with an increase in grazing severity (i.e., low post grazing residual sward height) and many studies have attributed the increased productivity of higher SR systems to an improvement in herbage utilization (McMeekan and Walsh, 1963). Penno (1999) suggested that the ideal stocking rate should balance the dual objectives of generous feeding to achieve high levels of production efficiency per animal and underfeeding to achieve high levels of pasture utilization to meet the overall objective of optimizing farm efficiency and profitability, while accounting for year-to-year variability in climate and grassland productivity. On that basis, intensified systems require grazing management practices that maximize pasture production and quality, which, in combination with increased stocking density, will result in increased overall system productivity (McEvoy *et al.*, 2009; Curran *et al.*, 2010; McCarthy *et al.*, 2013). A recent review of SR experiments reported a 0.20 increase in milk production per ha arising from a 1 cow ha^{-1} increase in SR, where no extra supplement was fed as SR increased (McCarthy *et al.,* 2011).

In addition to the economic and animal welfare benefits associated with grazing, grass-based ruminant livestock production systems provide an environmentally sustainable food production

model. In comparison with cropping, grassland is an important biological filter which reduces nutrient and chemical run off and supports biodiversity and carbon storage. Compared with arable land, grassland is associated with a better conservation of soil against erosion, and reduced runoff and leaching of nutrients into surface and ground water (Briemle and Elsasser, 1997; Jankowska-Huflejt, 2006). Grassland also acts as an important carbon sink for GHG emissions, due to its high organic matter content relative to arable land (Leip *et al.*, 2010). Notwithstanding these benefits, the efficiency of Nitrogen (N) use within grass-based systems is variable and can potentially result in nutrient loss to water resources by leaching. To comply with the Irish obligations pursuant to the EU Nitrate Directive (91/676/EEC), a long term study of the factors influencing nitrate loss beneath intensive dairy production systems in vulnerable soil types was undertaken at Curtins Farm, Teagasc Moorepark, in the south of Ireland over a 10-year period from 2001 to 2011. The Curtins Farm soil type is representative of the highest risk soils to nitrate leaching in Ireland. On the 48 ha site, cow numbers increased from 108 in 2001 to 138 in 2011, based on grazing management practices that increased grass growth and utilization which resulted in an increase in milk production from the site (Table 2).

Table 2. The effect of farm system characteristics on the biological efficiency of grass-based milk production at Curtins Farm (Teagasc, Ireland)

Year	2003	2005	2007	2009	2011
Stocking rate (cows/ha)	2.44	2.63	2.67	2.88	2.88
Grazing season (days)	293	295	306	287	285
Chemical N inputs (kg/ha)	289	331	313	248	249
Concentrate (kg/cow)	716	636	590	288	430
Milk volume ('000 L/ha)	15.6	15.5	14.6	14.4	15.3
Nitrate (NO_3^-N mg/l)	11.1	13.3	12.4	9.7	6.6

Best nutrient management practices were used on the farm to increase slurry-use efficiency and reduce fertilizer N application to the levels stipulated by legislation. Based upon detailed field-scale knowledge of soil capacity for nutrient retention, improved timing and rate of organic fertilizer application, reduced reliance on chemical fertilizer and the adoption of minimum-till cultivation reseeding, the result of this strategy was a consistent improvement in N-use efficiency and a decline in nitrate concentrations in groundwater at the site during the study period (Table 2).

In future, the sustainable intensification of animal production from pasture necessitates that management practices on the farm must be increasingly tailored to achieve excellent nutrient management outcomes in addition to productivity improvement. The results of this study indicated that intensive dairy production systems based on improved nutrient management and agronomic practices can quickly improve groundwater quality and lead to high water quality standards even on highly vulnerable free draining soils.

Combining high animal productivity with high feed self-sufficiency in grassland-based organic sheep production systems

The aim of organic farming is to establish and maintain soil-plant-animal interdependence and to create a sustainable agro-ecosystem based on local resources. Feed self-sufficiency and particularly forage self-sufficiency is therefore one of the fundamentals of organic farming.

To ensure sustainability in organic sheep production, the aim of one of the experiments conducted in the INRA Redon experimental site with a rustic breed (Limousine) and semi-natural grasslands with a stocking rate of 0.8 livestock unit/ha, was to optimize both ewe

productivity and self-sufficiency. This optimization was managed by i) fitting the lambing distribution to the seasonal dynamics of the vegetation resources, with 65% of the lambings in spring and 35% in autumn and adapting stocking rate to resource potential, ii) fattening lambs at pasture together with controlling parasitism level, iii) ensuring the provision of young forage of high quality for animals with high requirements (with increased use of legumes in swards), iv) practicing winter grazing for animals with low requirements, and v) sowing mixtures of cereals (triticale, barley and oats) and peas to increase feed self-sufficiency, with the aim of producing 40% to 50% of concentrate feed requirements (Prache *et al.*, 2011). In this way, feed self-sufficiency reached 95% in the last 3 years of the experiment. Moreover, combining a low reliance on bought-in concentrate feed with no mineral fertilization led to a very low use of non-renewable energy (51.0 MJ/kg lamb carcass, estimated using a Life Cycle Assessment approach; Pottier *et al.*, 2009) and low net greenhouse gases emissions (11.1 kg eq-CO_2/kg carcass; Prache *et al.*, 2011).

Conclusion

Sustainable intensification of grass-based ruminant production must ensure that the increased global demand for food is met in an economically and environmentally sustainable manner, producing a product which is acceptable to increasingly discernible consumers from a quality, social and ethical perspective. In this paper we have given examples showing that sustainable intensification of grass-based ruminant production allows both economic and environmental performance improvement at the farm level. At a broader level, other environmental issues such as carbon storage, biodiversity and landscape, and cultural issues must also be considered.

The key point which must be addressed is improving the efficiency of the animal-grass dynamic with fewer inputs and in a long-term sustainable manner. Further research should address the trade-off between quantity and quality in grass production, the resilience of the resource and its persistency over time. More systemic research on the animal-grass dynamic is needed to select and manage animals for grass and grass for animals. Future research should also address animal self-sufficiency, animals which are easy to manage and which are resistant to climate variation and parasites. In terms of management, sustainable intensification means changing from a position where the emphasis is on controlling all the management parameters to a position focused on compromising with risks and searching for equilibrium. Finally we should not forget that the production system is managed by the farmer. Helping farmers with management decisions through appropriate tools that combine grass, animal and system issues is a challenge for the greater development of grass-based systems.

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Theme 4 submitted papers

Plant or animal needs - how to determine the optimal N intensity of grassland?

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Abstract

The impact of six plant- and animal-related indicators for deriving the optimal N intensity of grassland was studied, based on a 3-year, multi-site field trial. Crude protein (CP) content was a limiting factor for grass-only diets, whereas for mixed rations, an appropriate share of maize could balance even high CP of grass, allowing the exploitation its yield potential.

Keywords: N fertilization, dry matter yield, N yield, crude protein, rumen N balance

Introduction

For high-yielding dairy cows dietary CP requirements are assumed to range between 14 and 18% (Pacheco and Waghorn, 2008). High CP content of grass silage may cause problems when paralleled by a high share of non-protein N, moderate energy concentration and if grass is fed as sole diet (Givens and Rulquin, 2004). If maize makes a significant portion of the diet the 18% CP threshold might be questioned and the N fertilizer recommendation might need reconsideration. The aim of the present study therefore was to derive the optimal N intensity of cut-only grassland when applying plant- and animal-related indictors.

Materials and methods

The study is based on a 2-factorial (site, N fertilization) randomized block experiment with 4 replicates conducted over three years (2009-2011) at five permanent grassland sites (sward age > 4 yrs, > 60% *Lolium perenne*) throughout Germany (Aulendorf, Baden-Württemberg; Eichhof, Hesse; Iden, Saxony-Anhalt; Riswick, North Rhine-Westphalia; Spitalhof, Bavaria). Nitrogen treatment comprised 6 levels (0 N-grass without clover; 0 N-white clover grass; 120, 240, 360, 480 kg N ha^{-1}) applied as calcium ammonium nitrate in four dressings, i.e. in early spring and after each of the first three cuts. Six indicators were investigated to derive the optimal N input (mineral N + N from fixation): (i) three dry matter (DM) response functions (linear-plus-plateau, quadratic-plus-plateau, exponential function; the latter with a marginal yield response of 10 kg DM (kg N)$^{-1}$), (ii) the CP content to represent the animal need, assuming an optimal range of 14-18%, (iii) the N yield, and (iv) the N uptake efficiency integrating crop and environment needs, with a target of unity, corresponding to a N surplus of zero. N fixation was estimated according to Hoegh-Jensen *et al.* (2004) as a function of DM yield, clover proportion and soil type. Forage quality traits were estimated by NIRS and rumen N balance (RNB) was calculated according to GfE (1997). The relationship between N input and indicator values were investigated by first estimating function parameters separately for each site×year×replicate combination and in a second step analysing the impact of site on a given parameter by an analysis of variance with site assumed fixed and year random.

Results and discussion

Maximum DM yield attained at the different sites was similar at 13-16 t DM ha^{-1} (Fig. 1). The tested DM response functions showed similar model fit (R^2 > 0.6). Thus no model could be favoured over any other.
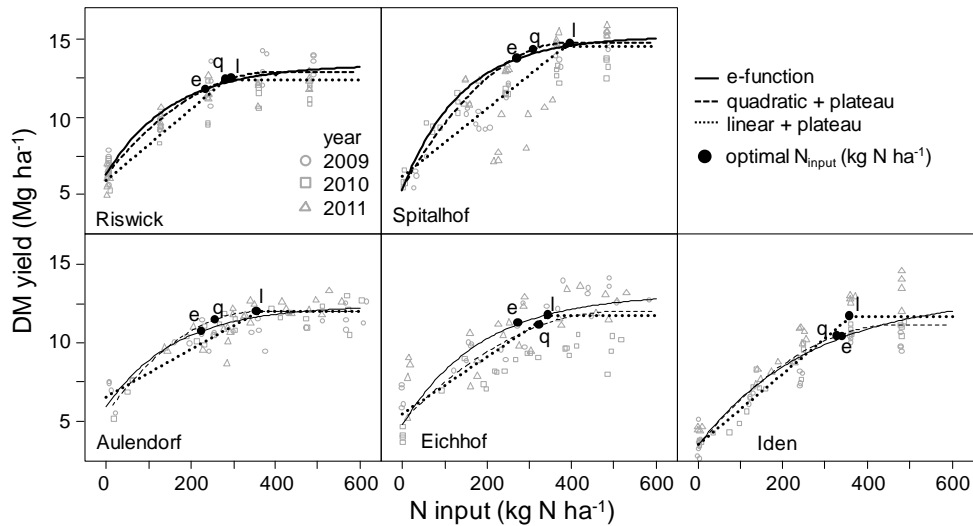
Figure 1. DM response functions obtained for the different grassland sites.

Depending on the function type, the optimal N input varied between 225 and 386 kg N ha^{-1}, with the e-function tending to estimate lower N input than the two others (Table 1).

Table 1. Optimal N input (kg N ha^{-1}, mineral N and N from fixation), depending on the site and indicator applied.

	Riswick	Spitalhof	Aulendorf	Eichhof	Iden
DM yield, linear-plus-plateau	284	386	360	345	359
DM yield, e-function and marginal yield	236	274	225	280	343
DM yield, quadratic-plus-plateau and marginal yield	279	313	258	324	328
N yield, linear-plus-plateau	354	388	348	427	424
N uptake efficiency	418	451	374	349	331
CP content (18% threshold)	155	149	32	199	223

Nitrogen yield increased linearly before reaching a plateau at an N input of 350 to over 400 kg N ha^{-1}. Correspondingly, the N uptake efficiency decreased almost linearly (data not presented). The N yield thus can be exploited only at a much higher N input than required for DM yield. Crude protein content showed a strong increase in N input and achieved weighted annual averages of up to 22%. The needs of the animal, as indicated by a CP content of 14-18%, thus could only be complied with when substantially reducing N input. This in turn would cause a considerable yield loss, e.g. 15-52% for the quadratic-plus-plateau function, and promote undesirable changes of sward composition. The DM yield potential was virtually exploited at an annual average CP content of 20%.

According to GfE (1997), a RNB of 50 is tolerable in the total ration. For dairy cows with moderate dry matter intake (13 kg DM d^{-1}) solely by grass silage with low CP content (grass silage 1, Table 2), a RNB of 50 could be complied with. The RNB threshold, however, will be exceeded substantially for grass silages with CP noticeably above 18% (grass silages 3 and 4, Table 2), resulting in increased N excretion and reduced N-use efficiency. The situation is entirely different if grass and maize silage are fed together, as exemplified in Table 2 for different grass/maize mixing ratios all achieving a RNB of zero. For grass silages with 16% CP a maize ratio of 30% is sufficient to balance RNB. With a higher maize share, as typical for many German dairy farms, grass silages with CP $\geq$ 20% can be compensated for.

Table 2. Protein value of model grass silages with varying CP contents and grass/maize rations. ME: metabolizable energy (MJ ME (kg DM)$^{-1}$), CP: crude protein (g (kg DM)$^{-1}$), RUP: feed CP escaping degradation in the rumen, RUPCP: RUP share (%) of CP, uCP : utilizable CP (g (kg DM)$^{-1}$), RNB: rumen N balance (g (kg DM)$^{-1}$). uCP: sum of microbial CP and RUP; RNB: ruminal N balance, RNB = (CP-uCP)/6.25

	Mixture	ME	CP	RUPCP	uCP	RNB
Grass silage 1		10.5	160	15	134	4.1
Grass silage 2		10.5	180	15	137	6.8
Grass silage 3		10.5	200	15	140	9.6
Grass silage 4		10.5	240	15	146	15.0
Maize silage		11.0	70	25	134	-9.8
GS1:MS	0.70 : 0.30					-0.1
GS2:MS	0.58 : 0.42					0.0
GS3:MS	0.50 : 0.50					-0.1
GS4:MS	0.35 : 0.65					0.3

The suitability of CP as indicator thus is depending on the ration. For high proportions of grass silage and low concentrate use, a threshold of 18% seems reasonable. When feeding diets with combinations of low grass but high proportions of maize and concentrates, higher grass CP contents can be easily compensated. When assuming such conditions for the tested sites, the optimal N fertilisation can be derived from one of the three DM response functions and the estimated N fixation. We used the quadratic-plus-plateau function since the functions did not differ statistically. With optimal N input ranging between 258 (Aulendorf) and 328 kg N ha^{-1} (Iden), and N fixation between 0 (Riswick) and 184 kg N ha^{-1} (Aulendorf), optimal N fertiliser input would range between 74 (Aulendorf) and 322 kg N ha^{-1} (Iden). Such grass swards require an efficient ensiling management in order to keep the protein quality on a high level. A crucial measure is a fast and extensive wilting, since protein degradation by plant proteases and microorganisms declines with increasing DM content (Edmunds *et al*., 2014).

Conclusion

The optimal N intensity of grassland varies substantially depending on the indicator applied. In terms of the availability of silage maize provided, high CP contents will cause little problems and the yield potential of grassland can be exploited. Thus, better use can be made of grassland as protein source. This needs refinement of fertilizer recommendations; in particular a greater differentiation is required with respect to the grassland use (grazing vs. cutting) and ration, as well as a better estimation of N fixation.

Acknowledgements

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Energy expenditure of two grazing Holstein cow strains

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Abstract

The study compared the energy expenditure (EE) of two Holstein cow strains in an organic grazing system. Twelve Swiss Holstein-Friesian (HCH) and 12 New Zealand Holstein-Friesian (HNZ) cows in the third stage of lactation were kept in a rotational grazing system without concentrate supplementation. Using the ^{13}C bicarbonate dilution technique in combination with an automatic blood sampling system, EE based on measured CO_2 production was determined during 6 h per d. The HCH cows were heavier and had a lower body condition score (BCS) compared to HNZ cows. Milk production and grass intake did not differ between the two cow strains, but HCH cows grazed for a longer time during the 6 h measurement period and performed more grazing mastication than the HNZ cows. No difference was found between the two cow strains with regard to EE per metabolic body weight ($BW^{0.75}$), mainly due to a high between-animal variation. As efficiency and energy use are important in pasture-based dairy systems, the determining factors for EE and its variation, respectively, should be investigated in more detail in future studies.

Keywords: Energy expenditure, pasture, dairy cows, Holstein-Friesian

Introduction

The economic benefit of pasture-based dairy systems requires the efficient use of pasture herbage and is also reasonable milk production per cow (Dillon *et al*., 2005). By limiting herbage allowance to improve the efficient use of pasture herbage per area, the herbage intake, and therefore the energy uptake per cow would be reduced (Delagarde *et al*., 2001). On the other hand, EE under grazing conditions is increased. For example, Bruinenberg *et al.* (2002) found that grass-fed dairy cows in the barn have a 10% higher metabolizable energy requirement for maintenance. Physical activity also influences EE, e.g. grazing cows had 21% higher EE compared to grass-fed cows kept indoors (Kaufmann *et al*., 2011). The energy requirements relative to maintenance may increase up to 50% depending on grazing conditions, including herbage availability (allowance and mass) and digestibility, distances walked (to the milking parlour and watering points), weather, topography, and interaction between these factors (CSIRO, 2007).

In New Zealand, Holstein cows are bred for the specific needs of pasture-based, low-input dairy production, including selection for milk solids, lower body weight (BW), fertility, and longevity (Miglior *et al*., 2005). Compared to Swiss Holstein cows, the NZ Holstein cows had a lower BW, showed different BCS, ruminated longer, and tended to take more steps on the pasture (Schori and Münger, 2010). The objective of the current study was to determine EE based on CO_2 production of HNZ cows and heavier, high-producing HCH cows in an organic, full-time grazing system without concentrate supplementation. To explain possible differences in EE between strains with differences in grazing behaviour or physical activity, these variables were recorded simultaneously.

Materials and methods

The experiment took place on the organic farm 'Ferme de l'Abbaye' in Sorens (824 m a.s.l., Switzerland) on the second half of September 2011. Twelve matched pairs of HCH and HNZ cows were formed according to the following criteria: number of lactations (2.6 ± 1.8), weeks in milk (28 ± 0.6 week), and age for primiparous cows (35 ± 3.9 month). All 24 experimental cows were separated from the rest of the lactating herd and received no hay or concentrate supplementation. Access to fresh water, minerals and common salt were provided. The experiment consisted of two consecutive experimental weeks. Cow pairs were equally divided between the 2 weeks so that each cow underwent a period of 7 d of data collection. Cows grazed from 08 to 14 h and from 18 to 4.30 h. The paddocks were long-established swards, predominantly of grasses, and were rotationally grazed based on sward height measured with an electronic rising plate meter (Jenquip, Feilding, NZ) for 1 to 3 d. The average pre-grazing sward height was 6.4 (±0.7) cm, and the average post-grazing sward surface height was 4.4 (±0.6) cm. During the experiment, the average temperature was 15.4 (min. 9.3, max. 18.1) C. Milk yield (7 d) and composition (3 d), BW (7 d), BCS (before and after the experiment) were measured during the respective experimental week. Individual dry matter intake (DMI) was estimated using the n-alkane double-indicator technique. Grazing and ruminating behaviour was recorded on 3 consecutive d using an automatic jaw-movement recorder with a pressure sensor (Datenlogger MSR145, MSR Electronics GmbH, Hengart, Switzerland). Physical activity, including time spent standing, lying and walking, and numbers of steps, were determined on 3 d using the IceTag[TM] pedometer (IceRobotics Ltd., Edinburgh, UK). The ^{13}C bicarbonate dilution technique combined with an automatic blood sampling system was used to determine EE (Kaufmann *et al.*, 2011). Between 07.45 and 13.45 h the CO_2 production of one cow pair was measured. Data were analysed using a linear mixed model (SYSTAT 12).

Results

The HCH cows had a greater BW (615 vs. 567 kg, $P = 0.01$) but a lower BCS (2.54 vs. 2.84, $P = 0.01$) and showed the same BW losses (96 vs. 112 g/d, $P = 0.91$) compared to HNZ cows. Neither milk yield (18.8 vs. 17.5 kg, $P = 0.31$) nor energy corrected milk yield (ECM; 18.3 kg, $P = 0.96$) differed between the cow strains. The milk fat (4.01 vs. 4.49 %, $P = 0.03$) and protein (3.26 vs. 3.65, $P = 0.001$) content were lower for HCH than for HNZ cows. Similar amounts of DM (16.5 vs. 16.3 kg/d, $P = 0.89$) were consumed. The production efficiency measures, namely ECM produced per 100 kg of $BW^{0.75}$ (14.8 vs. 15.7 kg, $P = 0.25$), ECM produced per kg of DMI (1.12 vs. 1.13, $P = 0.85$), and DMI per 100 kg of $BW^{0.75}$ (13.3 vs. 14.0 kg, $P = 0.41$) were not affected by cow strain. During the 6 h of blood sampling for EE determination, HCH cows spent more time grazing (235 vs. 213 min., $P < 0.001$) and performed more grazing mastication (17514 vs. 15634, $P = 0.001$) than the HNZ cows. Ruminating behaviour did not differ between the two cow strains. Cow strain had no effect on time spent standing (280 vs. 281 min., $P = 0.92$) lying (80 vs. 79 min., $P = 0.92$) or on time spent walking (109 vs. 95 min., $P = 0.34$) and the number of steps (1186 vs. 1106, $P = 0.43$). Cow strain did not affect EE per kg $BW^{0.75}$ over 6 h (309 vs. 273 kJ, $P = 0.27$). HCH were heavier, but EE per cow per d (152 vs. 127 MJ, $P = 0.13$) did not differ between cow strains.

Discussion

Energy expenditure measured with the ^{13}C bicarbonate dilution method includes the metabolizable energy costs for maintenance (MEm, including energy for fasting metabolism and activity allowance, measured under thermoneutral conditions), as well as heat increment changes (heat losses, concrete differences between metabolizable energy and net energy for maintenance, production, and gestation). Although HNZ cows, in contrast to HCH cows, were selected indirectly for feed-conversion efficiency by considering BW in the breeding worth,

and thus intake capacity, as well as MEm in relation to the production of milk solids, equal ECM per kg DMI and EE per kg BW$^{0.75}$ per d values were found. Possible reasons could be that, compared to earlier studies (Schori and Münger, 2010), an increasing similarity in the BW of the two cow strains was found. Furthermore, the variability of EE between animals was high (CV = 26%) and clearly higher compared to a CV of 17% as observed by Kaufmann *et al.* (2011). The larger variation may be explained by the use of different cow strains, the larger variation of the length of the path to the paddocks, and the topography of the pastures in the foothills.

Under controlled activity conditions, Van Es (1961) found that the MEm of cows of the same breed and similar size may vary by as much as 8-10%. It is assumed that a major contributor to the variation in fasting EE is the genetically inherited amount of organ mass. Furthermore, Van Es (1961) discussed not only the between-animal variation but also analytical and physiological variation, as well as variation in the composition of rations as additional effects that can contribute to the variation in MEm. Moreover, under practical grazing conditions, variability is even greater compared to respiration chambers since, among others, herbage availability and quality, climate, and the physical activity of dairy cows also varies.

Conclusion

Similar production levels in late lactation, small differences in BW and physical activity of the two Holstein cow strains led to similar values of EE per kg BW$^{0.75}$ in both strains. The high variability suggests that there is potential to improve the efficient use of consumed energy. Furthermore, the determining factors for EE of grazing cows should be investigated in more detail.

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Effects of mechanically separated dairy cow slurry on grazing performance

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Abstract

Inorganic fertilizers are widely used to fertilize grasslands in dairy systems. Increasing the nutrient-use efficiency of slurry applied as an alternative or supplementary fertilizer could reduce the volume of purchased fertilizer. This would reduce costs and improve security of future fertilizer supply since slurry is produced on-farm. Slurry separation could increase the potential for fertilizing grazed grassland with slurry. This experiment compared the effects on milk yields of fertilizing grazing swards with the liquid fraction of separated slurry, whole slurry or inorganic fertilizer. Three groups of 12 core cows were grazed for two 24-day rotations on 2-day paddocks between 18 and 24 days after fertilizer treatment application in a put-and-take design. Nutrient-use efficiency was significantly higher ($P<0.05$) from the liquid fraction of separated slurry (12.2 kg kgN^{-1}) compared to whole slurry (8.0 kg kg N^{-1}) application. This effect was most likely due to a combination of improved soil infiltration and reduced sward contamination.

Keywords: slurry separation, grazing, dairy

Introduction

Increasing yields from grazed grasslands using sustainably sourced fertilizers represents a major opportunity if we are to sustainably maintain, or even increase levels of food production. Currently, slurry can be applied to grazed grasslands as a fertilizer, increasing the availability of essential plant nutrients (e.g. nitrates and ammonium) in the rhizosphere. However, inorganic fertilizers continue to be applied by farmers, since slurry contains a high proportion of dry organic matter which becomes assimilated into soils more slowly than inorganic fertilizers, resulting in lower grass yields (Møller *et al.*, 2000). Separation of slurry into the liquid fraction prior to application may therefore benefit farmers by reducing dry matter (DM) content and increasing rates of soil infiltration; hence increasing herbage yields due to greater concentrations of nutrients being available to grass roots in solution (Møller *et al.*, 2000). Reducing the DM content may also enhance the potential for using slurry on grazing ground due to the lower sward contamination of more liquid slurries (Rodhe, 2003), reducing the risk of sward rejection by grazing cattle. This is of interest given that cattle grazing performance can be adversely affected by slurry applications on grazing pasture (Gjestang *et al.*, 1984). Previous research investigating the use of slurry on grazing ground has shown that applying slurry by shallow injection can improve the resultant grazing performance relative to splash-plate application (Laws *et al.*, 1996). Likewise, Dale *et al*. (2012) showed that inorganic fertilizer inputs can be reduced by replacing a portion with cattle slurry applied by trailing-shoe without adversely affecting dairy cow performance. This experiment compared the effects of applying separated slurry or unseparated slurry (without the addition of inorganic fertilizer) or inorganic fertilizers on grass yields, milk yields and nutrient-use efficiency.

Materials and methods

The experiment was conducted during the summer of 2013 at the SRUC Dairy Research Centre, Dumfries, Scotland (UK Ordnance Survey map ref NX981732). Three 1-ha paddocks were

established in each of four fields. Two of the fields were dominated by perennial ryegrass (*Lolium perenne*) and two were dominated by Italian ryegrass (*Lolium multiflorum*) (approx. coverage of dominant species greater than 95%). Each paddock in each field was then allocated one of three fertilizer treatments: whole (unseparated) slurry (W), liquid fraction of separated slurry (L) and ammonium nitrate fertilizer (F). Each treatment paddock was further subdivided into three 0.33 ha sub-paddocks with the aim of each sub-paddock providing two days grazing for twelve cows (with a herbage allowance of 15 kg DM cow-day^{-1}). All fields were cut for silage in late May. Fields 1-3 were grazed initially on the silage aftermath and Field 4 was cut and baled before grazing. Dairy cow slurry was separated using a Sperrin dual cylinder separator and applied using a dribble bar approach at 24 m^3 ha^{-1} (liquid) and 27.5 m^3 ha^{-1} (whole). The different slurry rates, and an ammonium nitrate control, were calculated so that equal concentrations of available N (NO_2^- + NO_3^- + NH_4^+) were added to each treatment, according to standard values (Defra, 2010). Three groups of twelve mid- to late-lactation dairy cows were grazed on the treated pasture for two 24-day rotations, under a three-times-a-day milking regime, with 0.5 kg cow^{-1} of concentrates being fed at each milking. Additional cows of a similar yield and weight were added to the groups when required to provide a target herbage availability of 15 kg DM cow-day^{-1}. Statistical analysis was carried out using R i386 3.0.2 (R Core Team, 2013). Differences between the composition of W, L and F were tested using student's t-tests. Treatment differences for herbage yield, milk yield and nitrogen-use efficiency (yield / available N) were tested using Analysis of Variance tests (ANOVA) and Tukey's Honestly Significant Difference (HSD) tests.

Results and discussion

Slurry separation decreased the mean DM content of the slurry from 52±0.8 g kg^{-1} for W to 28±0.3 g kg^{-1} for L *(P<0.001)*, but also reduced the mean concentration of available N from 1.3±0.02 g kg^{-1} to 1.1±0.01 g kg^{-1}, respectively (*P<0.001*), contradicting the standard values used to calculate the application rates (1.2 g kg^{-1} for W and 1.5 g kg^{-1} for L). The L fraction was therefore applied at a lower total rate over the experiment and had a lower concentration of available N, resulting in available N application rates of 68.9, 50.2 and 71.8 kg ha^{-1} for W, L and F, respectively. The stocking densities were 36.4±1.6 LU ha^{-1} for W, 36.4±1.4 for L and 45.8±1.8 LU ha^{-1} for F (*P<0.001*), showing that more grass was grown by F even though it was balanced with W and L by available N.

Daily milk yields were not affected by treatment at the cow or area (stocking density x yield) level (*P>0.05*). This suggests that grazing has not been adversely affected by using slurry as a fertilizer, regardless of whether it has been separated or not. However, the nutrient-use efficiency (yield / available N applied) was significantly affected by treatment (Table 1). For every kg of available N applied, L produced 52% more milk than W. Greater productivity is most likely due to improved soil infiltration resulting in a greater concentration of nutrients available to roots in solution for L than W, coupled with lower sward contamination for L than W, in line with the findings of Rodhe (2003). The lower rate of available N application for L than W may account for some of the increase in efficiency, due to the non-linearity of N response curves. However, had the available N been equal between the treatments and the increased efficiency remained, there may have been a significant effect of separation on milk yield at the area level.

Table 1. Mean daily milk and fat and protein yields. W = whole (unseparated) slurry, L = liquid fraction of separated slurry, F = ammonium nitrate fertilizer

	W		L		F		sig.
	Mean	s.e.	Mean	s.e.	Mean	s.e.	
Daily milk yield (kg cow^{-1})	15.1	0.2	16.8	1.0	16.0	1.5	NS
Daily fat + protein yield (kg cow^{-1})	1.14	0.02	1.19	0.07	1.13	0.10	NS
Daily milk yield by area (kg ha^{-1})	550	8	612	35	733	66	NS
Daily milk yield by available N (kg kgN^{-1})	8.0[a]	0.1	12.2[b]	0.7	10.2[ab]	0.9	*

NS, not significant; *$P<0.05$.

Conclusions

The L treatment increased nutrient-use efficiency relative to W. This may be due to higher nutrient availability from L than W due to increased soil infiltration and smaller particle size. However, care needs to be taken in extrapolating these results since L was applied at a lower rate. Daily milk yield per cow was not affected by treatment; hence grazing performance does not appear to have been reduced by using either separated or whole slurry as a fertilizer relative to inorganic fertilizer. The results demonstrate the potential for growing grass for grazing from slurry alone, and suggest that separation may have a place in certain systems although more work is required to establish the economic implications.

Acknowledgements

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Effect of growth stage on the phosphorus content of grass, and on phosphorus excretion on dairy farms

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Abstract

To protect the environment in the Netherlands, legal limits for P and N application in manure and mineral fertilizer have been established. Because phosphorus (P) in grass silage is an important source of P excretion on dairy farms in the Netherlands, decreasing the P content in grass can prevent manure export costs. The P content of grass decreases when it is cut at a later growth stage. However, feeding values also decrease, resulting in decreasing milk production or increasing use of manufactured feed. It is hard to predict the final effect of cutting grass at a later growth stage on P excretion at the farm level. Therefore we quantified the relation between growth stage and P content of grass and P excretion at the dairy farm level. The relation between P content of grass and growth stage was determined from the results of three grassland experiments. This relation was used in simulations with three farms in the farm model DairyWise. The P intake in roughages of dairy cows decreased when grass was cut at a later stage, but intake of manufactured feed increased. The largest decrease of manure export was reached at zero grazing (100% grass silage): 4.4 m^3 ha^{-1} manure ($\approx$ €66 ha^{-1}). On all farms mowing costs decreased about €70 ha^{-1} to €115 ha^{-1}.

Keywords: phosphorus, grassland, P content, excretion, growth stage, simulation

Introduction

In regions with intensive animal husbandry, excessive use of phosphorus (P) and nitrogen (N) in the past has led to negative effects on the environment. To protect the environment in the Netherlands, legal limits for P and N application in manure and mineral fertilizer have been established, based on excretion standards. Dairy farmers have the possibility to prove lower excretion of their cattle by analysing and measuring roughage stocks. Because P in grass silage is an important source of P excretion, decreasing the P content in grass can prevent manure export costs. It has been shown that the P content of grass decreases when it is cut at a later growth stage (Wilson and McCarric, 1967; Fleming and Murphy, 1968; Whitehead, 2000). Feeding value, however, in terms of energy and protein content, also decreases. At the farm level, a result of cutting at a later growth stage could mean decreasing milk production, or increased use of manufactured feed. Because milk production and the feeding ration influence P excretion, it is hard to predict the final effect at the farm level of cutting grass at a later growth stage. The objective of this study is to quantify at the dairy-farm level, the relation between growth stage and P content of grass, and the effect on excretion, of cutting grass at a later growth stage than usual.

Materials and methods

Between 1999 and 2007 three grassland experiments, nine experimental years, took place (Table 1). The experiments were originally not designed for our study but provided useful data. In the experiments, dry matter (DM) yield and P content were determined every two weeks. The cuts were taken from new plots with the same treatments, in two replicates. The sward was dominated by *Lolium perenne*. Data were statistically analysed with Restricted Maximum Likelihood (ReML) technique (Harville, 1977), using Genstat (v.15). ReML fits a random and a systematic model to the data. The starting model included soil type, number of growing days,

N and P fertilization, starting day of growth and all interactions. Random model included experiment and year. Non-significant interactions ($P \leq 0.05$) were deleted.

Table 1. Grassland experiments to quantify the relation between P content and growth stage, range of N and P fertilization, and dry matter yield per cut.

Experiment	Exp years	Soil type	Treatments	No. of records	N fertilization	P$_2$O$_5$ fertilization	DM yield
					Range per cut, kg ha^{-1}		
1	1999 2000	Sand, Clay	Mineral P fertilization, +/– dairy manure, growth stage	1120	0-150	0-50	60-7700
2	2002 2003	Peat	Mineral N fertilization, growth stage	1728	0-90	0-80	170-10000
3	2005 2006 2007	Peat	Mineral N fertilization, groundwater level, growth stage	1495	0-120	0-45	65-10000

The developed statistical model was included in the farm model DairyWise (Schils *et al.*, 2007) which simulates technical aspects and economics of dairy farms. Technical aspects are: grassland fertilization, growth, quality and use (grazing and mowing), consumption by the dairy herd and milk production. In the model the relation between feeding values of grass and growth stage is quantified. The economic results are calculated from costs and benefits. Using DairyWise, three model farms were calculated (Table 2), with cutting at two growth stages: normal (N: 3 t DM ha^{-1}) and late (L: 4 t DM ha^{-1}) cuts were taken for silage. Cuts for grazing and milk production were not varied.

Table 2 Characteristics of three model farms

Farm	Farm area (ha)	Grazing method	Additional feeding during grazing period	Feeding during housing period	Milk production (t ha^{-1})
1	60.0	Day and night	0	100% grass silage	14.3
2	55.7	Day	8 kg DM/day maize silage	50% grass silage – 50% maize silage	15.4
3	60.0	zero	---	100% grass silage	14.3

Soil type: sand. Milk production per cow: 8600 kg yr^{-1}

Results and conclusions

In the statistical analysis of the grassland experiments it was proven that an effect of cutting grass at a later growth stage was a lower P content. The P content on a certain harvest date was higher if the N fertilization was higher. On peat the P content was highest, on clay lowest. Calculation with the model, using estimated factors, showed that in, e.g., a cut that started to grow at 1st March, on sand, fertilized with 120 kg N ha^{-1}, the decrease of P content was about 0.028 g P kg^{-1} DM day^{-1}. In the same situation, the decrease on peat was 0.035 g P kg^{-1} DM day^{-1} and on clay it was 0.014 g P kg^{-1} DM day^{-1}. On sand fertilized with 80 kg N ha^{-1} the decrease was 0.025 g P kg^{-1} DM day^{-1}.

In calculations with the model Dairy Wise (Figure 1) results showed that the P intake of dairy cows that was obtained from roughages decreased on all farms when the grass was cut at a later growth stage. Part of this decrease, however, was compensated with an increased intake of P from manufactured feed. The largest decrease was reached on farm 3 (zero grazing, 100% grass silage): 1.2 kg P yr^{-1} cow^{-1}. At the farm level, this was equal to a decrease of 4.4 m^3 ha^{-1} manure export ($\approx$ €88 ha^{-1}). On farm 2 (50%-50% maize-grass in ration) the P excretion was already

low in the normal situation, and the effect of cutting at a later growth stage was small. On all farms, cutting at a later growth stage decreased the mowing costs by about €70 ha^{-1} on farms 1 and 3, and by €115 ha^{-1} on farm 2. Another effect of cutting at a later growth stage on farm 3 was that grazing pressure was higher as the swards required for the mowing cuts took more time to grow.
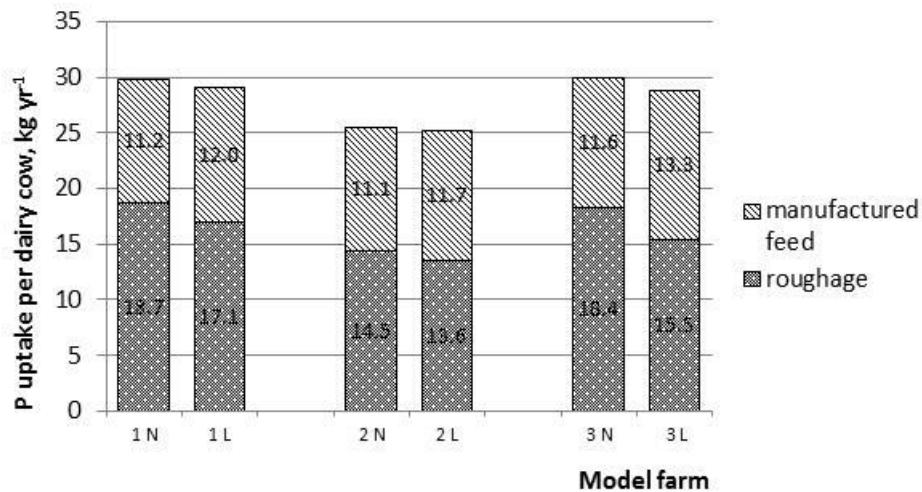
Figure 1. Phosphorus intake of dairy cattle (kg P cow^{-1} year^{-1}, milk production 8600 kg yr^{-1} cow^{-1}) on three model farms at normal (N) and late (L) cuts.

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Effects of concentrate levels on milk production and traffic of cows milked by a mobile automatic milking system on pasture

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Abstract

Cows milked by an automatic milking system in pastures were assigned to two groups receiving different amounts of concentrates (2.1 kg vs 4.1 kg). The effect of level of concentrates on milk yield (MY) and returns to the robot was assessed. Level of concentrates had a positive influence on daily milk production over the grazing period, as cows of the low-concentrates group produced 21.43 ± 0.62 kg compared with 24.33 ± 0.62 kg in the high-concentrates group. However, this effect was modulated subsequently by grass quality and availability. Regarding daily voluntary returns to the robot, the high-concentrates group showed higher frequency (3.66 ± 0.05, compared with 3.22 ± 0.04 in the low concentrates group) demonstrating a positive impact of concentrate supply on cow traffic.

Keywords: grazing, dairy cows, concentrate, automatic milking system, milk performance

Introduction

The use of an automatic milking system (AMS) implies there will be a stimulation of cows' traffic to the AMS. Several studies showed discrepancies about the effects on cows' traffic of the supply of concentrates distributed at milking; some of them finding no effect on milking frequency (Bach *et al*., 2007; Jago *et al*., 2007) while in more recent studies (Lyons *et al*., 2013) returns to the robot seem to be improved by supplementary feeding. The aim of this study was to analyse the influence of concentrates on milk yield and returns of grazing cows milked by an AMS in pasture.

Material and methods

The study was conducted from 1 May to 30 September 2013 at the Experimental Farm of Sart Tilman, University of Liège. Only those animals present from the beginning until the end of this period were taken into account. These were divided randomly into two groups each of which received a different level of concentrates. Fifteen cows, including 7 primiparous, days in milk (DIM): 97 ± 63 days, with an average lactation number (NL) 2.00 ± 1.25, were assigned to the low-concentrates (LC) group, while the high-concentrates group (HC) included 14 cows, of which 5 were primiparous (DIM = 94 ± 41 days; NL: 2.43 ± 1.91). From 1 May to 30 October the cows' diet was based on grass except for a variable amount of concentrate distributed in the AMS at milking. As grass availability decreased in September, a mixture of maize silage, dried beet pulp, alfafa pellets and straw, giving a total amount of 8 kg DM per day and per cow, was provided as a supplement from 11 September until the end of the study period. The cows were milked by an AMS Lely A3next® which was on a trailer in order that it could be moved onto pastures following the procedure described by Dufrasne *et al.* (2012). Transponders fixed on the HR-tag neck collar (SCR, Israel) were used in order to identify the cows and to register several parameters: milk yield (MY), number of milking, number of milking failures, and the amount of concentrate given and milking time. Twenty-four ha of pastures, which consisted mainly of perennial ryegrass and white clover, were divided into 15 plots ranging from 0.6 to 3.1 ha, and the maximum distance for the cows to walk to the robot of 700 m. Cows were assigned to different plots for day and night. The change from day to night plot was managed when cows came out the AMS, as they were driven by selection gates to their new allocation.

Strip-grazing was achieved in order to provide cows with fresh grass every day. Animals stayed during 4.6 ± 1.5 days on each plot (min. = 1d; max.= 12 d). Grass height was measured using a herbometer (Jenquip®) when the cows came in and out, and the change from one parcel to the next one was decided on this basis. Grass cover was estimated by mowing a grass strip of 10 m length, and by weighing the mown sample (kg DM). Samples were analysed by NIRS to determine their nutritional value. Daily parameters (MY, number of milkings, kg concentrates per milking, nbr returns per cow calculated by summing the milkings, refusals and failures) were analysed over the grazing period. Influence of month, parity, lactation stage and their interactions was analysed using GLM procedure (SAS. 91).

Results

Grass mean heights at entrance were 9.3 ± 3.0 cm and 6.2 ± 1.0 cm at exit (Table 1). Grass digestibility declined over the grazing season (Table 2). Lignin content was highest in August, while the water soluble carbohydrate content was the lowest. This could be linked to weather conditions (dry with high temperatures).

Table 1. Grass height at entrance and exit over the grazing period

	Grass height in (cm)	Grass height out (cm)	Difference (cm)
May	16.3	6.8	9.5
June	10.3	5.8	4.5
July	12.8	6.7	6.1
August	11.0	5.9	5.1
September	9.9	5.6	4.3

Table 2. Evolution of grass nutritional values during grazing period (g kg DM^{-1})

Month	DM	Crude protein	Lignin	Water soluble carbohydrate	Digestibility (%)
May	17.5 ± 2.3	162.0 ± 32.6	27.29 ± 3.20	186.6 ± 32.5	82.29 ± 4.15
June	17.0 ± 4.3	175.3 ± 40.1	26.88 ± 2.30	184.5 ± 42.4	83.63 ± 5.42
July	17.6 ± 4.1	180.5 ± 31.8	36.00 ± 4.60	141.3 ± 38.1	77.33 ± 5.89
August	20.9 ± 4.8	193.7 ± 44.5	43.29 ± 3.55	97.6 ± 26.4	79.71 ± 4.82
September	19.1 ± 6.2	206.1 ± 39.8	39.00 ± 2.55	126.9 ± 20.7	78.78 ± 5.04

Table 3. Effect of concentrates on milk yield and voluntary returns to the robot over the grazing period

Month	Milk yield (kg) mean ±SE		Difference in MY per kg concentrate (kg kg^{-1})	Nbr returns cow[-1] d^{-1} Mean ±SE	
	LC	HC		LC	HC
May	26.1 ± 0.7	26.3 ± 0.7	0.04	2.9 ± 0.1[a]	3.1 ± 0.1[b]
June	21.5 ± 0.8[a]	24.3 ± 0.7[b]	1.19	3.4 ± 0.1[a]	3.8 ± 0.1[b]
July	22.8 ± 0.7[a]	25.5 ± 0.7[b]	1.37	3.0 ± 0.1[a]	3.4 ± 0.1[b]
August	19.7 ± 0.7[a]	24.0 ± 0.7[b]	2.43	3.4 ± 0.1[a]	4.0 ± 0.1[b]
September	17.1 ± 0.7[a]	21.8 ± 0.7[b]	3.21	3.4 ± 0.1[a]	4.0 ± 0.1[b]

LC and HC denote low concentrates and high concentrates, respectively. Means within a row with different letters superscripts differ ($P<0.001$).

In both the LC and HC groups the MY decreased over the grazing time with the increase in number of days in milk (Table 3). In May, LC and HC cows produced the same amounts of milk. This absence of response to the distribution of concentrates can be explained by the high availability of good quality grass. Difference in milk production between the LC and HC groups increased from 2.69 kg in June to 4.66 kg in September. The impact of the level of concentrates was more pronounced when grass availability and/or its quality was poor, as occurred at the end of the grazing season or in August when weather conditions were unfavourable for grass growth and grazing. The distribution of the mixture 'maize silage, beet pulp and alfalfa pellets' did not prevent milk production from decreasing. On average, the HC group produced 2.9 kg more milk over the season, representing 1.5 kg of milk per kg concentrate. Returns to the robot were more frequent in the HC group, whatever the month.

Conclusion

The response of milk yield to concentrates at grazing can vary considerably during the grazing season. In this trial, milk yield was generally improved by higher supply of concentrates. When grass availability and quality decreased, the difference between the high-concentrates and low-concentrates groups was more important. In this trial, the level of concentrates had a positive influence on the cows' traffic to the robot.

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Relationship between fatty acid content and nutritive value of perennial ryegrass (*Lolium perenne*)

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Abstract

There is growing interest in creating novel forages to address consumer demands, such as enhancing product quality and functionality and reducing environmental impacts of ruminant production. To address these, one area under investigation is the fatty acid content of forages. This paper focuses on the relationship between total fatty acid (TFA) content and other nutritional characteristics of perennial ryegrass. A study was set up involving twenty-four genotypes from two populations of perennial ryegrass. Genotype selection was based on historic soil plant analysis development (SPAD) data, with the intention of selecting a set of genotypes that best represent the variation in TFA content of each population. Plants were maintained in a polytunnel for three months prior to harvesting. Fatty acids were quantified using gas chromatography (GC) while CP and WSC were predicted using near-infrared reflectance spectroscopy (NIRS). Total fatty acid content was found to correlate positively with CP ($R^2 = 0.69$, 94 d.f., $P<0.001$) and negatively with WSC ($R^2 = 0.14$, 94 d.f., $P<0.001$).

Keywords: *Lolium perenne*, fatty acids, water soluble carbohydrate, crude protein

Introduction

Plants are the primary source of n-3 fatty acids, both in marine and terrestrial ecosystems, due to their unique capability of *de novo* synthesis of 18:3n-3. This fatty acid is the building block for longer-chain n-3 series of essential fatty acids, via elongation and desaturation pathways (Barceló-Coblijn and Murphy, 2009). Although grass has a somewhat low total fat content, with the lipid content of leaf tissue varying between 3-10% of DM (Hawke, 1973), the majority of this comprises of the PUFA 18:3n-3 and 18:2n-6. Thus, it can contribute greatly, and in some situations wholly, to the total lipid intake of ruminant animals. Dietary lipid content and composition contributes towards 1) energy provision to the animal and 2) the fatty acid profile of ruminant products (i.e. meat/milk). With respect to improving the fatty acid profile of ruminant products, increased emphasis has been placed on the potential of using plant-based strategies of late (Morgan *et al.*, 2012). Grass is a high PUFA feed source which is cheap, natural and environmentally sustainable, thus is a noteworthy strategy to improve fat composition of ruminant products (Dewhurst *et al.*, 2003). Studies have highlighted a strong genetic basis to fatty acid composition (Dewhurst *et al.*, 2001). Hegarty *et al.* (2013) have successfully identified regions of the genome which are associated with fatty acid composition of perennial ryegrass. However, large genotype × environment interactions have also been recognized (Dewhurst *et al.*, 2003; Palladino *et al.*, 2009).

Materials and methods

Four control genotypes were selected from an Aurora × AberMagic F1 mapping population and twenty experimental genotypes selected from the B674G intermediate-heading 13th generation breeding population. Genotypes were selected in order to provide the best possible representation of the variation in fatty acid content within each population, based on historic Soil Plant Analysis Development (SPAD) data which have been shown to correlate positively with fatty acid content (Morgan *et al.*, 2013). Mature single ryegrass tillers were transplanted into 15-cm pots in potting compost during April 2012. Four replicate clones of each genotype

were used and were arranged in a randomized block design. Plants were maintained in a polytunnel, with actively reproductive heads cut back every two weeks in order to encourage tillering. Plants were harvested by hand in July 2012 to a cutting height of 5 cm. All plant material was collected and temporarily stored on ice during harvest then freeze-dried and stored at -20°C. Near infrared reflectance spectroscopy (NIRS) analysis was carried out as described by Lister and Dhanoa (1998) to estimate WSC and CP (= N × 6.25) content. Fatty acid content was determined via the procedure of Sukhija and Palmquist (1988) using Tricosanoic acid methyl ester (C23:0) as an internal standard (Sigma Aldrich Co, St. Louis, MO, USA). Fatty acid methyl esters (FAME) were separated and quantified using gas chromatography (GC-FID) system (CP-3800 with PAL Autosampler, Varian Inc, CA, USA) equipped with a CP-select 100 m × 0.25 mm chemically bonded for FAME column (Agilent technologies UK Ltd, Berkshire, England, UK). Peaks were identified using a 37 FAME standard (S37, Supelco, Poole, Dorset, UK) and quantified using the C23:0 internal standard. Data were analysed via Genstat (14th edition; VSN International Ltd, Hemel Hempstead, UK) using linear regression and correlation.

Results and discussion

Total fatty acid (TFA) content ranged from 14.5 to 34.0 g kg^{-1} DM with a mean value of 23.3 g kg^{-1} DM. These values are similar to those published by Dewhurst *et al.* (2001, 2002) and Van Ranst *et al*. (2009) for July harvests of perennial ryegrass. Crude protein content ranged from 6.9 to 20.1% DM and WSC content ranged from 7.9 to 34.6% DM, with mean values of 13.3% DM and 18.8% DM, respectively. A very strong, positive relationship was found between TFA and CP ($R^2 = 0.69$, $P<0.001$), which is illustrated in Figure 1. Other studies which investigated the relationship between N or CP and fatty acid content of forages have also found positive relationships. Elgersma *et al.* (2005) found a very strong relationship between CP and C18:3n-3 ($R^2 = 0.90$, $P<0.001$) with similar results for CP and TFA relationship, while Boufaied *et al.* (2003) reported a positive relationship between TFA and N content ($R^2 = 0.79$, $P<0.001$). Crude protein and WSC are known to have an inverse relationship (Humphreys, 1989). Consequently, the relationship between TFA and WSC (illustrated in Figure 2) was also found to be negative ($P <0.001$); however it is worth highlighting that this was found to not be a strong relationship ($R^2=0.14$).
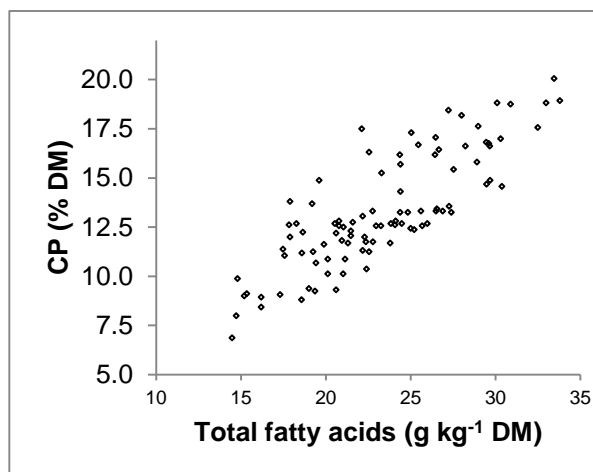

Figure 1. Relationship between crude protein content (% DM) and total fatty acid content (g kg-1 DM) of perennial ryegrass
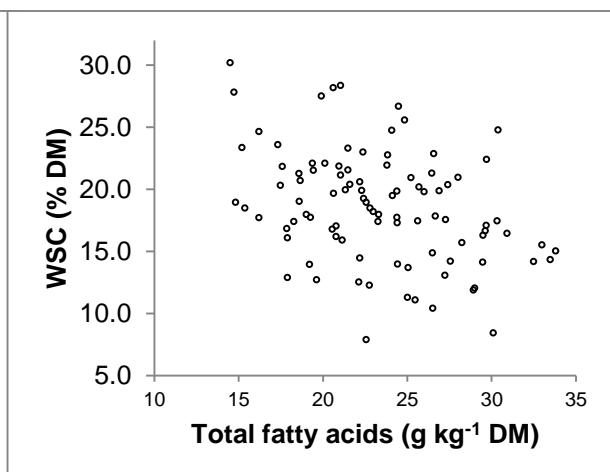
Figure 2. Relationship between water soluble carbohydrate content (% DM) and total fatty acid content (g kg-1 DM) of perennial ryegrass

Conclusion

This study provides further testimony to the positive relationship between TFA and CP. Deeper exploration of this relationship is needed to provide insight into the mechanisms underpinning it. This study has also uncovered the relationship between TFA and WSC. Further research is needed to confirm this relationship and to assess if it is similar across a wider range of varieties/species and growth season/environmental conditions

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Can we use the fatty acid composition of bulk milk to authenticate the diet composition?

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Abstract

The aim of this work was to predict cow diet composition from the fatty acid (FA) concentrations of bulk milk collected in ten different EU countries. A dataset comprising 1248 bulk cow-milk samples and corresponding information related to diet composition assessed by rapid interviews with farmers was used. The predictions based on FA for cow diet composition were excellent for authenticating the proportions in cow diets of fresh herbage ($R^2 = 0.81$), good for hay, intermediate for maize silage and grass silage ($R^2 > 0.62$) and poor for concentrates ($R^2 < 0.51$). The prediction models we presented could offer a valuable tool to authenticate cow feeding.

Keywords: milk fatty acid, feeding system, authentication

Introduction

In several recent studies, plant biomarkers (such as terpenes, polyphenols, carotenoids), stable isotopes, and milk fatty acid (FA) were proposed as candidate compounds in milk for the analytical authentication of animal diets. The milk FA composition was the more effective method to discriminate cow diets including low or high amounts of maize silage into the diet (Engel *et al.*, 2007). Indeed, cow diet is known to be the most important driver for FA composition (Chilliard *et al.*, 2007; Coppa *et al.*, 2013). However, only few studies have tested the potential of bulk-milk FA profile to predict the composition of cow diets adopted by commercial farms and these studies were made at a large territorial scale (Engel *et al.*, 2007). The aim of this work was to predict cow diet composition from FA concentrations of bulk milk collected in different EU countries.

Materials and methods

The FA profiles of 1248 bulk cow milk samples and their related production conditions were compiled from a selection of 20 published or unpublished studies carried out from 2000 to 2010 in ten EU countries: France, Germany, Italy, Norway, Slovakia, Slovenia, Czech Republic, Denmark, Sweden and the Netherlands. The detailed composition of this dataset is given by

Coppa *et al*. (2013). It included milk collected on commercial farms from a latitudinal range of 44° N to 60° N, from sea level to 2000 m altitude, from 13 different cow breeds and in all seasons. To develop a model of prediction of diet composition based on milk FA profile, a general linear model was applied using experiment as fixed factor and FA concentrations as covariates.

Results and discussion

The fresh herbage proportion in cow diets was reliably predicted by the model ($R^2 = 0.81$). The FA explaining most of the model variability were iC17:0 and C18:2t11c15, both linearly and positively related to fresh herbage proportion in the cow diet. The C18:2t11c15 is an intermediate of ruminal biohydrogenation of dietary C18:3n-3, which is the main herbage FA (Chillard *et al*., 2007), and its increase in milk with increasing proportion of fresh herbage in the cow diet is well known (Couvreur *et al*., 2006; Chilliard *et al*., 2007). Vlaemink *et al*. (2006) showed a linear increase of iC17:0 with increasing of the major bio-hydrogenation intermediates, such as C18:2trans11cis15. In our model, fresh herbage proportion in cow diets also decreased linearly with C16:0, increased linearly with C17:1c9 and decreased cubically with C17:0 concentration in milk. An increase in proportion of fresh herbage in cow diets was previously associated with an increase in C17:0 in milk and with a decrease of C16:0 (Couvreur *et al*., 2006).

The prediction model for maize silage proportion had a lower fit *($R^2 = 0.66$)* when compared to the fresh herbage model. Most of the model variability was explained by the C16:1c9+aiC17.0 to iC16:0 ratio, linear increase of concurred with increase in proportion of maize silage in cow diet. High values of this FA ratio were also associated by Engel *et al*. (2007) with a diet rich in maize.

Most of the variability of the prediction model for hay proportion in cow diet ($R^2 > 0.74$) was explained by milk iC14:0, C18:3n-3 concentrations and C18:2t11c15 to C18:1t10+t11 ratio. The positive quadratic relation between milk iC14:0 and hay proportion in the cow diet can be easily explained by the microbial origin of this FA, which is derived from ruminal cellulolytic bacteria (Vlaemink *et al*., 2006). The positive linear relation we found between C18:3n-3 concentration in milk and hay proportion in cow diet is in agreement with Chilliard *et al*., (2007). This trend agrees also with the negative linear relation we found in the ratio of C18:2t11c15 to C18:1t10+t11 (Chilliard *et al*., 2007).

The model fit for grass silage proportion was similar to those of maize silage ($R^2 = 0.62$). Grass silage proportion in cow diets decreased linearly with increasing milk isoC17:0 concentration. This trend is in agreement with findings of Vlaemink *et al*. (2006) which showed a decrease in this FA when grass silage was replaced by maize silage in cow diets. The C12:0 and C15:0 were negatively and positively quadratically related to grass silage, respectively. A decrease in C18:2c9t13 was linearly related to an increase in grass silage concentration, confirming the lower concentration of the intermediates of ruminal biohydrogenation of ingested C18:3n-3, when fresh herbage is substituted by grass silage (Chilliard *et al*., 2007).

The lowest reliability of FA for the authentication of animal diets was found for the prediction of the proportion of concentrates in cow diets ($R^2 < 0.51$). The FA explaining most of the model variability were iC14:0, C18:2n-6, and the C16:1c9+aiC17:0 to iC16:0 ratio. The concentrate proportion in cow diets decreased with linearly decreasing isoC14:0, and increased with quadratically increasing of the C18:2n-6 concentration in milk. These results are in agreement with the FA profile of milk derived from diets poor in forages (Chilliard *et al*., 2007). A linear decrease in C16:1c9+aiC17:0 to iC16:0 ratio was associated with an increase in concentrate proportion in cow diets in our model, in agreement with Vlaemink *et al*. (2006).

Table 1. Prediction models of proportions in cow diets of various feeds, based on milk FA composition. The FA are listed according to their decreasing weight in model determination (RMSE: root mean square error)

Ingredients (% of DM diet)	Fatty acid	n	RMSE	R^2
Fresh herbage	iC17:0;C18:2t11c15;C17:1c9;C17:0^3;C16:0;C18:2n-6;C18:2t11c15^3; C17:1c9^3;C16:0^3;C18:2c9t13^2;C18:1t10+t11^3;iC16:0^3	693	15.6	0.81
Maize silage	C16:1c9+aiC17:0;C18:3n-3;C20:5n-3;C18:3n-3^2;C20:4n-6;C15:0; C18:3n-3^3;C15:0^2;C18:2n-6^3;C15:0^3; C18:2t11c15/C18:1t10+t11; C18:1c9+t13^3;C18:2c9t13^3;C18:2n-6	678	9.1	0.66
Hay	iC14:0^2;C18:2t11c15/C18:1t10+t11;C18:3n-3;C18:3n-3^2;C16:0; C17:0^2;C15:0;C17:0;C18:2c9t13;C18:1t10+t11;C14:0^2;C20:4n-6; C14:0;C20:5n-3;C20:4n-6^3	683	11.2	0.75
Grass silage	iC17:0;C12:0^2;iC17:0+C16:1t9^3;C15:0^2; C18:2c9t13;iC14:0; C16:1c9+aiC17:0/iC16:0;C18:2t11c15/C18:1t10+t11;iC16:0;C18:2n-6^3; iC16:0^3;C18:2n-6;C16:0;C16:0^3;C16:1c9+aiC17:0;C20:4n-6	659	10.6	0.62
Concentrates	iC14:0;C16:1c9+aiC17:0;C18:2n-6^3;C17:0;iC14:0^3;C18:1t10+t11; C17:0^3;iC17:0+C16:1t9^2;C18:3n-3^2;C18:3n-3;C18:2c9t13^3	883	8.1	0.51

Conclusion

Our work provided original and reliable models to predict cow diet composition based on milk FA composition. A dataset composed of bulk milk collected in several European countries was used. These prediction models could offer a valuable tool to authenticate cow feeding.

Acknowledgements

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Effect of dietary supplementation on milk production and milk composition of grazing dairy cows in late lactation

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Abstract

Grass silage is now recommended as an alternative to concentrate to supplement the grass-based diet of dairy cows in late lactation. The aim of this experiment was to establish the effect of supplementation on the milk production of grazing dairy cows in late lactation. Eighty-four spring-calving dairy cows were randomly allocated to one of six treatments: high grass-only allowance (HG), low grass-only allowance (LG), grass with low concentrate (GCL), grass with low grass-silage (GSL), grass with high concentrate (GCH) and grass with high grass-silage (GSH). Treatment GCH had a greater milk yield than all other treatments, and GCL had a greater milk yield than GSH, GSL, HG and LG. There was no difference between the milk yield of GSH, HG and LG; however, the milk yield of the GSL treatment was greater than on HG and LG. Milk solids yield was greater in the two concentrate-supplemented treatments than in the other four treatments, and LG had the lowest milk solids yield. Milk fat concentration was lower in the silage-supplemented treatments and in GCH than in HG. Milk protein concentration was significantly higher in the two grass-only treatments than in the four supplemented treatments. Concentrate supplementation in late lactation increased milk solids yield compared to grass-only or grass supplemented with silage. However, supplementation with either concentrate or silage negatively affected milk fat and protein concentration.

Keywords: supplementation, nutrition, milk yield, milk composition, silage, concentrate

Introduction

Dairy farming in northern European temperate countries, such as Ireland, is based on spring-calving and the efficient conversion of grazed grass into milk (Dillon *et al*., 1995). The objective of the system is to allow grazed grass to make up as large a proportion as possible of the diet of the lactating dairy cow (O'Donovan *et al*., 2004). Grass supply is often limited in the spring and autumn, and so at this time the predominantly grass-based diet is supplemented. Concentrate feeds are commonly offered in the late lactation period in order to meet the requirements for maintenance, growth, lactation and pregnancy. More recent recommendations are that supplementation could be in the form of grass silage. Grass silage is the next most important ruminant feedstuff after grazed grass (McEniry *et al*., 2011) and is a cheaper alternative to concentrate (Finneran *et al*., 2010). There are few comparisons in the literature of concentrate and grass silage as supplementary feeds in late lactation. Therefore, the aim of this experiment was to investigate the effects on milk production of offering grass silage or concentrate supplementation to grazing dairy cows in late lactation.

Materials and methods

Eighty-four spring-calving lactating dairy cows were blocked on milk yield, milk composition, parity, bodyweight, body condition score and EBI. From within block, the cows were randomly allocated to one of six treatments: high grass-only allowance (HG), low grass-only allowance

(LG), grass with low concentrate allowance (GCL), grass with low grass-silage allowance (GSL), grass with high concentrate allowance (GCH) and grass with high grass-silage allowance (GSH). Treatments HG and LG were allocated 17 and 14 kg DM grass per cow/day respectively. Treatments GCL and GSL were offered 14 kg DM grass per cow/day and 3 kg DM supplementation per cow/day. Treatments GCH and GSH were offered 11 kg DM grass per cow/day and 6 kg DM supplementation per cow/day. The average DMD of the silage was 697 g/kg and it was offered after morning milking. The concentrate offered was a 160 g/kg crude protein dairy concentrate and was offered in two equal amounts at morning and evening milking. Milk yield was recorded daily and milk composition was recorded weekly. Data were analysed using covariate analysis and the PROC GLM statement of SAS. Treatment, week of experiment and the appropriate covariate were included in the model.

Results and discussion

Table 1 shows the milk production performance of the study.

Table 1. Effect of dietary supplementation on milk production and milk composition of grazing dairy cows in late lactation.

	Grass only		14 kg DM grass + 3 kg DM supplementary feed		11 kg DM grass + 6 kg DM supplementary feed			
	17 kg DM (HG)	14 kg DM (LG)	Concentrate (GCL)	Silage (GSL)	Concentrate (GCH)	Silage (GSH)	S.E.	*P-Value*
Milk yield (kg/d)	11.9[a]	11.6[a]	14.8[b]	12.8[c]	16.0[d]	12.1[ac]	0.96	<0.001
Milk fat (g/kg)	57.9[a]	57.7[ac]	57.0[ac]	55.0[bc]	55.1[bc]	54.5[b]	1.30	<0.01
Milk protein (g/kg)	42.1[a]	41.6[a]	39.9[b]	40.1[b]	39.8[b]	39.7[b]	0.50	<0.001
Milk solids (kg/d)	1.16[b]	1.06[a]	1.40[c]	1.21[b]	1.54[c]	1.12[b]	0.030	<0.001

[a-d] Means within a row not sharing a common superscript differ significantly (*P*<0.05)

Milk yield was significantly (*P*<0.001) affected by treatment. Treatment GCH had a greater milk yield than all other treatments, and GCL had a greater milk yield than GSH, GSL, HG and LG. There was no difference between the milk yield of GSH, HG and LG; however, the GSL treatment had a greater milk yield that HG and LG. The milk yields of HG and LG agree with the study of Reid *et al*. (2013). The increase in milk yield when late lactation grazing cows were supplemented also agrees with that study. Milk solids yield was greater in the two concentrate supplemented treatments than in the other four treatments, but did not differ between the two concentrate supplemented treatments. This indicates that there was no advantage in offering 6 compared to 3 kg DM concentrate in late lactation to grazing dairy cows. There was no difference in milk solids yield between HG, GSL and GSH, all of which were greater than LG. Milk fat concentration was significantly lower in the silage supplemented treatments and in GCH than in HG. The negative effect on milk fat concentration of silage supplementation in late lactation was previously measured (O'Brien *et al*., 1996) and the reasons for this should be explored in more detail. The negative effect of high concentrate supplementation on milk fat concentration was not unexpected as high levels of dietary concentrate can be associated with a reduction in rumen pH and consequent reduction in milk fat concentration (Abrahamse *et al*., 2008). There was a negative effect of supplementation on milk protein concentration, which agrees with previous research (Sutton *et al*., 1996) and which suggests a negative effect on milk processability (O'Brien *et al*., 1996).

Conclusion

This experiment shows that in late lactation, supplementing a grass-based diet with concentrate increased milk solids yield compared to feeding grass only or grass supplemented with silage. Supplementation with either silage or concentrates negatively affected milk fat and protein concentration. The effects of supplementing grass-based diets in late lactation on milk processability should be measured.

Acknowledgments

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Combining robotic milking and grazing

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Abstract

In 2013 in France, nearly 3000 milk producers use an automatic milking system (AMS). It often leads to reduced grazing, for fear of a drop in milking frequency. A national project was set up to bring technical solutions to breeders who wish to combine AMS with grazing. During 5 years, the experimental farm of Derval tested several traffic options and herd management opportunities while grazing. The maize-silage silo was closed for 34 days (in 2012) and 56 days (2013). Holstein cows on a 100% grazed diet produced, on average, 27.5 kg of milk with 2.8 kg of concentrates. The feeding cost was one-third the winter ration. Each cow ingested up to 1.5 t of grazed grass per year. During the same programme, 20 French robotic farms were surveyed with various saturation levels, grass growth potential and traffic. This programme shows that grazing with an AMS remains possible as long as the farmer keeps motivation, a sufficient grazeable area, and implements the right traffic options.

Keywords: AMS, grazing, milking frequency, dairy cows

Introduction

In France, the massive introduction after 2000 of AMS often led to reduced use of grazed grass in the diet of dairy cows (Jégou *et al*., 2007; Billon, 2009). The possible decrease in milking frequency which is often presented as an important factor for individual productivity often threatens the breeders. As they are also seeking to reduce working time, grazing does not always appear as a solution to reach this target, but keeping grazed grass in cow diets is advocated for its positive impacts on nutrition and health (Burow, 2011). It is also an efficient solution to the need to reduce production costs thanks to the low cost of per energy unit to produce milk from grazed grass compared with other feeds. The experimental farm of Derval (western France) tested different solutions to graze dairy cows milked with an AMS. The research programme had associated experiments in stations as well as pilot farms in order to broaden the list of solutions that can be used to reconcile AMS and grazing.

Materials and methods

The experiments lasted from 2009 to 2013. The following aimed at studying the impact of the share of grazed grass in the daily diet ("day grazing only", or "day and night grazing") on the performances of the cows and the robot, compared to the performances during the winter period when the cows are kept inside. The average number of cows (Holstein) milked by the robot (a Delaval one-stall VMS 2007) reached 72. The yearly production is ca. 9000 kg of milk /cow and there are ca. 150 milkings every day. The cow traffic is guided: the cowshed equipped with cubicles has a drafting gate only usable from the feeding area. After the robot, a second gate directs the cow towards grazing or towards an isolation box. Derval is located in a dry area (mean 600 mm rainfall /year). The grazable area reaches 28 ha of temporary grasslands of ryegrass-white clover. One large track (3.5 m) leads to the 3 paddocks of 10, 10 and 8 hectares. Grass management is a simplified rotational system. The maximal walking distance for the cows to reach the end of the furthest paddock is 800 m. One water trough is located at the entrance of the shed and a second just before the exit. No water is available in the fields. The buffer feed (maize silage) is adjusted to the amount of grass outside and the growth forecasts to maintain 10 days of grass ahead. It is delivered in the morning. When the cows get 8 kg DM per day, they do not have access to the trough as long as the whole herd is not inside the shed,

to ensure all the cows have the same access time to the buffer feed. The amounts of grass grazed were estimated through the difference between the calculated Intake capacity of the cows (INRA 2007, R Delagarde, pers. comm.) and the intakes of supplements. SAS ® software was used to analyse data with PROC MEANS, GLM and MIXED. For weather data, classification methods (PCA and HAC) were used. A number was dedicated to each feeding period: P1 (100% shed period), P2 (transition period) and P3 (100% grazing period.) Statistical analyses are based on 2011, 2012 and 2013. The international production cost method (IFCN) was used to assess the feeding cost and the margin over feeding cost.

Results

The access time to grazing allowed by the traffic management led to the valorization of more than 1 ton of grazed grass per cow per grazing season. In 2013 the maize clamp was closed for 56 days. The individual dairy production remained around 27.5 kg during the 100% grazed grass diet both in 2012 and 2013 (Table 1).

Table 1. Dairy production, milking frequencies and concentrates per period.

	2011			2012			2013		
	P1 61d	P2 44d	P3 11d	P1 60d	P2 74 d	P3 32d	P1 60 d	P2 33d	P3 56d
Cow number	68	74	74	73	74	74	69	71	67
Lactation stage (d)	206	178	178	192	189	185	226	229	232
Milk kg d^{-1} c^{-1}	27.6	30.4	28.3	29.4	31.6	27.6	30.1	30.7	27.5
Diff to P1	/	+2.8	+0.7	/	+2.2	-1.8	/	+0.6	-2.6
Concentrates kg c^{-1} d^{-1}	4.8	3.9	2.7	3.9	3.9	2.3	5.6	4.6	3.3
Diff to P1	/	-0.9	-2.1	/	0	-1.6	/	-1.0	-2.3
Milk kg per AMS d^{-1}	1876	2253	2097	2130	2323	2044	2074	2182	1842
Milking frequency c^{-1} d^{-1}	2.15	1.86	1.97	2.06	1.92	1.86	2.12	2.08	2.12
Diff to P1	/	-0.3	-0.2	/	-0.14	-0.2	/	-0.04	0

During P3, concentrate (wheat) delivered is limited to 2.8 kg c^{-1} d^{-1} versus 4.8 average during P1. Adjusted means of the productions are 28.9 kg d^{-1} c^{-1} for P1, 30.3 for P2 and 27.5 for P3. An increase of 1.4 kg c^{-1} d^{-1} occurred during P2 ($P< 0.0001$). During P3, there was a decrease of 1.4 kg compared to the average production during the winter period P1 ($P< 0.0001$), partly due to a decrease by 1.6 to 2.3 kg c^{-1} d^{-1} of concentrates in 2012 and 2013. The average milking frequency ranges from 1.86 to 2.15 milkings c^{-1} d^{-1} in relation to the saturation level of the robot, though variation between P1 and P3 is only a decrease by -0.3 to -0.04 milkings c^{-1} d-^{1}. The adjusted mean for milking frequency is 2.11 milkings c-^{1} d^{-1} in P1. This frequency decreases in P2 (1.96 milkings c^{-1} d^{-1}) then reaches 1.99 milkings c^{-1} d^{-1} in P3. Thus, the effect of grazing leads to a decrease by 0.15 milkings c^{-1} d^{-1} ($P<0.0001$) in P2 and only by 0.11 milkings c^{-1} d^{-1} ($P<0.0001$) in P3.

The weather influences the return of the cows from pasture (Lozach, 2011). Days with the lowest milking frequency (1.79 milkings c^{-1} d^{-1}) are characterized by high wind speed with high humidity, with no effect of rainfall. In terms of working time, during P2, sorting cows takes ca. 5 min to check milking times on computer, and 10 min to sort cows inside the shed. This is done daily at ca. 8 am after delivering maize silage, so cows are brought to feed racks. During P2 and P3, the cows are fetched by the herdsman at ca.6 pm, 20-40 min. are required. In terms of economic impact, the feeding cost for 1000 L delivered decreases from 148 € in P1, to 43 € in P3. Thanks to grazing, the monthly margin over feeding cost (Figure 1, curve with dots) remains over 200 € per 1000 L whatever the seasonal effect of the milk prices.

Figure 1: feeding cost and margin over feeding cost.
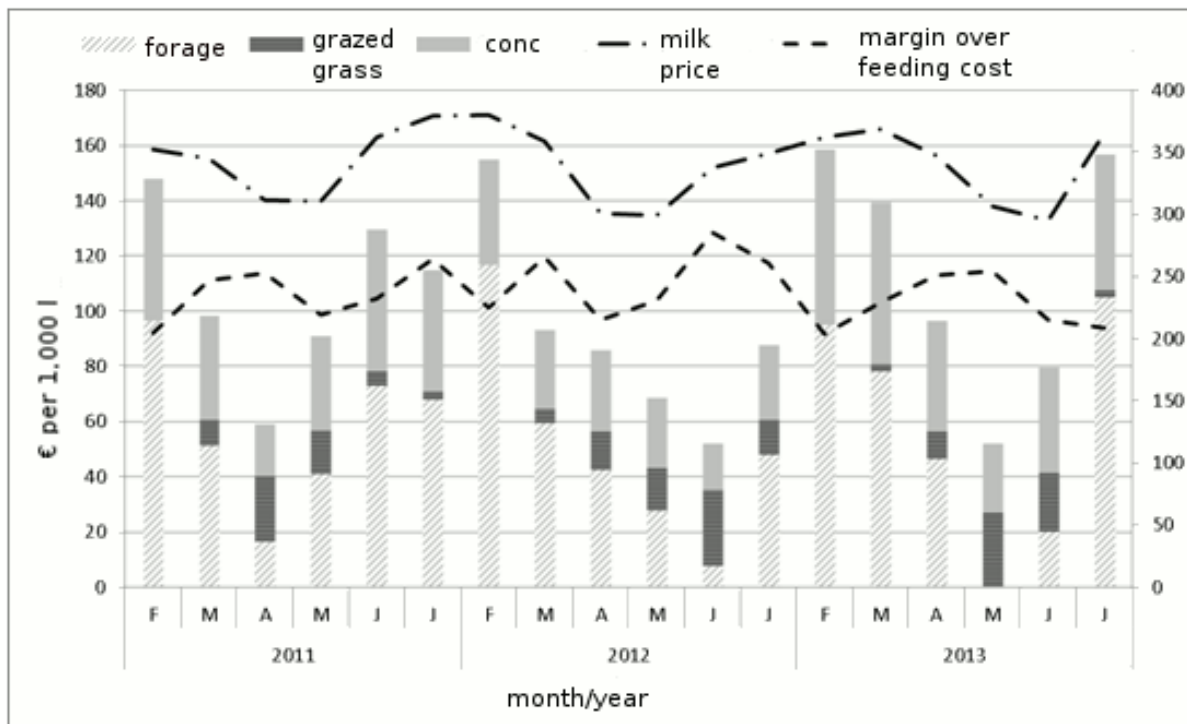
Discussion

The results show the same tendencies as on the 20 pilot farms followed during 3 years within the CASDAR research programme (Carles, 2013). On those farms the milking frequency decreased by 0.24 milkings c^{-1} d^{-1} with a drop by 1.7 kg of milk d^{-1} during the grazing period. The amount of grazed grass in Derval reaches the same level as on the 20 pilot farms (average of 1500 kg DM c^{-1} y^{-1}; range 750 to 2600), and 1500 kg DM is exactly the average grazed grass intake by the average French dairy cow (Brunschwig, 2011).

In terms of working time, two tasks are directly related to grazing: at 8 am the cows milked between midnight and 6 am are sorted out, and in the evening the herd has to be fetched from the field. Using two paddocks per 24 hours (day and night paddocks) could facilitate the separation by the farmer of cows already milked from those not milked (Oudshoorn, 2008) but this requires two daily interventions to empty the night- and day-paddocks. To avoid fetching the cows, a system with 3 paddocks per 24 h like in Ireland or New-Zealand (Fitzgerald, 2012, and Woolford, 2004) could also be implemented.

Conclusion

The Derval experiment with a robot at full capacity, together with results from pilot farms, clearly show that grazing (even 100% grazing with no silage) can be combined with robotic milking. The feeding cost was reduced to one third: this limited the negative effect of the fluctuations of the milk price in spring. The same tendency was shown in the pilot farms. The success of the system lies in the trust of the cows in terms of traffic. Cows have a strong capacity to adapt to any traffic solution as long as we give them enough time to do it.

Acknowledgements

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GPS tracking of Old Norwegian ewes on a coastal heathland-dominated island

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Abstract

Coastal heathland is an endangered habitat type, which in Norway is maintained by grazing with Old Norwegian sheep. The breed is well adapted to graze the habitat type all year round. While there is excess of feed during the summer, the availability of winter feed can be a challenge, and in some years additional feed is necessary. In this paper, we evaluate the diet selection of Old Norwegian ewes grazing a coastal heathland-dominated island in Northern Norway during June/July, September and January/February. Twenty-eight ewes were equipped with GPS-collars and their position registered every six hours. Faeces samples from the ewes were collected during the three periods and analysed using micro-histology. In addition, the island was vegetation mapped. The three data sets allow us to suggest which species the ewes graze and how they utilize the pasture of the island. We conclude that during summer there is enough available feed for the 28 ewes, whereas during winter we expect the island to be over grazed.

Keywords: native sheep, micro-histology, diet selection

Introduction

The Old Norwegian breed is the native breed of Norway and belongs to the group 'Northern short-tailed breeds'. The breed is most common in coastal heathland habitats of Norway. The European coastal heathlands are considered an endangered habitat type (EU Habitat Directive 92/43/EEC) threatened by climate change, air pollution, land-use change and abandonment (Moen *et al.*, 2006). There are special needs to protect the coastal heathland from becoming extinct as a habitat type. Grazing this habitat with Old Norwegian sheep has traditionally been one of the most important methods for maintenance. The sheep graze all year round and the challenge in this system is available winterfeed. Old Norwegian sheep are better adapted to the climate and poor vegetation during winter than domesticated breeds and their metabolism allows the animals to feed on woody forage like leaves, twigs and bark. In Norway, shelter is demanded for animals kept outdoors during winter and, in cases of feed shortage, the animals must have access to additional feed (Lovdata FOR-2005-02-18-160). There are few observations on natural diet selection during winter. Norderhaug and Thorvaldsen (2011) found that *Calluna vulgaris* and *Carex* sp are important species during the winter. In this paper, we evaluate the diet selection of Old Norwegian ewes grazing a coastal heathland-dominated island in Northern Norway during June/July, September and January/February.

Materials and methods

The island, Risvaer, is located in the Luroey community in Northern Norway (66.3°N, 12.6°E). The island, 48 ha, was vegetation mapped in 2011 following the classification system by Fremstad (1997). Seven permanent plots (1×1 m) were established for registration of species composition and their cover (%). Twenty-eight Old Norwegian ewes grazed the island from June 2012 until June 2013. The ewes were equipped with GPS-collars (Telespor®) logging their position every six hours. Kernel-Density maps were estimated on the points for each of three periods (June/July 2012, September 2012 and January/February 2013). Fresh faeces samples from 9, 6 and 10 ewes were collected in June, September and January, respectively,

for micro-histological analyses. The samples were analysed using the Garcia-Gonzalez method (Garcia-Gonzalez, 1984). Vegetation types and GPS-data from June/July, September and January/February (Figure 1) were compared on maps using ArcMap 10.1. Micro-histological analyses were compared against the maps to explain the different grazing patterns during the year.

Results and discussion

Risvaer is dominated by species-rich heathland vegetation. Usually, *Calluna* is the dominating species in coastal heathlands. However, under rich soil conditions, as in the southern part of the island, herbs and graminoides represent a considerable proportion at the expense of *Calluna*. In contrast, *Empetrum nigrum, Vaccinium uliginosum* and *Calluna* are dominating the heathland in the northern part of the island. *Juniperus communis* is also an important species at Risvaer. Abandoned, former cultivated grassland is the second-most important vegetation type dominated by graminoids such as *Phleum pratensis*, *Agrostis* spp. and *Carex nigra*, depending on variations in the soil moisture content. A shelter for the animals is placed in this area. We compared the GPS-data with the vegetation and the faeces samples. The GPS-data indicate where the ewes preferred to be during the three periods presented in this paper. Comparison of the three data sets allows us to suggest how the ewes utilize the pastures at Risvaer and which species they graze at different times of the year.
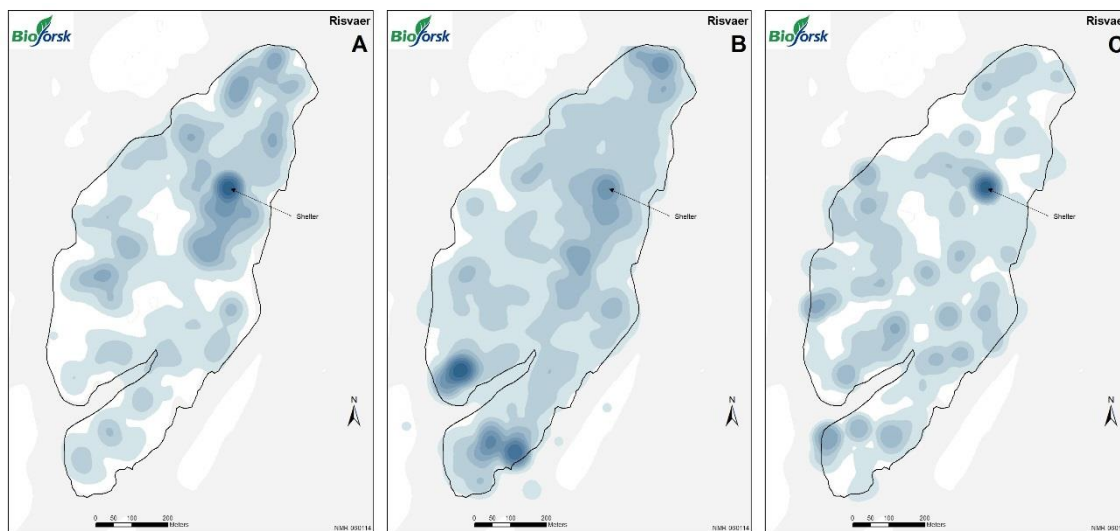
Figure 1. Kernel-Density map showing position of ewes in (A) June/July 2012, (B) September 2012 and (C) January/February 2013. The darker spots indicate more ewes staying in this area.

The map for June (Figure 1A) shows that the ewes stayed mainly on the grass-dominated area early in the summer, in order to find the first green shoots from *Poaceae* and herbs, which are high in nutrients. During summer the ewes spread over the island (Figure 1B) without any preference in type of vegetation; this is in contrast to winter (Figure 1C) when the ewes moved less and were found mainly in the heathland vegetation south at the island or around the shelter. The most abundant species found in the faeces analyses are presented in Table 1. The analyses do not take into account digestibility. Hence, differences in species portion can only be compared between different sample times but not within the same sample. However, analyses of faeces samples show that graminoids and herbs were important in June. In September, graminoids dominated the diet with a decline of *Carex* spp and herbs compared to June. In January, however, the diet changed considerably, and *J. communis* and *Carex* spp. seemed to be important. *Juncus communis* is a species that is rarely grazed due to its low nutritional value and high fibre content. *Calluna*, on the other hand, is considered an important species for Old Norwegian sheep during winter (Norderhaug and Thorvaldsen, 2011). Since this species is

scarcely represented at Risvaer, we suggest the intake of *J. communis* to be compensating for the lack of *Calluna*.

Table 1. Average proportion (%) of micro-histological material in faeces samples for important species in June 2012, September 2012 and January 2013.

	June 2012 (N = 9)	September 2012 (N = 6)	January 2013 (N = 10)
Herbs	20.5	7.9	5.4
Graminoids (total)	78.1	89.4	63.9
Festuca spp.	46.1	67.7	29.7
Deschampsia flexuosa	4.8	5.5	1.0
Arrenatherum elatius	1.8	1.4	0.2
Agrostis spp.	2.8	1.4	1.2
Molinia caerulea	2.7	0.3	0.6
Carex spp.	12.8	9.6	29.4
Heather			
Calluna vulgaris	-	0.6	1.2
Cypress			
Juniperus communis	0.2	-	26.5

On the other hand, the preference of *J. communis* can indicate overgrazing during winter. In January, the high proportion of *Carex* spp. in the faeces samples is in accordance with Norderhaug and Thorvaldsen (2011) and *Carex* are considered important species during winter. *Carex* spp. were grazed in the heathland and not in the boggy areas of cultivated grassland where *Carex* spp are the dominating species. Possibly, these areas are too wet during winter, which is why the ewes prefer to graze the species in the drier heathland areas in the southern part of the island.

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Nutritive value of leaf fodder from the main woody species in Iceland

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Abstract

In the past, leaf fodder from woody species played an important role as animal feed in Iceland. However, very limited information exists on the nutritive value of the main woody species used as fodder. The aim of our study was therefore to determine forage quality of leaves of *Betula nana, B. pubescens*, *Salix lanata*, *S. phylicifolia* and *Sorbus aucuparia* and to compare it with forage quality of a common native grass, *Deschampsia cespitosa,* and with an introduced grass, *Alopecurus pratensis,* used by contemporary Icelandic farmers for forage production. In late June 2013, we collected samples of all species at four localities in Iceland and determined concentrations of nitrogen, phosphorus, neutral and acid-detergent fibre and lignin and compared them with the optimum range required for sheep nutrition. Nutritive value of leaves of woody species was relatively high and browsing of their leaves and collection of their leaf fodder for winter feeding could satisfy sheep/cattle nutritional requirements for N and P. However, the high content of indigestible lignin, present in all the woody species, functions as a barrier to nutrient digestibility. Grasses were characterized by lower P. The forage quality of leaves of woody species increased in the order *B. nana < B. pubescens < S. phylicifolia < S. aucuparia < S. lanata*.

Keywords: livestock feeding, forage quality, North Atlantic Isles

Introduction

In northern regions, human subsistence in the past was almost exclusively based on animal products. The colonization of the North Atlantic islands, including Iceland, by Norse settlers (AD 800 – 1000) was thus connected with the spread of livestock; namely cattle, sheep, horses and goats (Amorosi *et al*., 1997). Livestock browsing was, together with wood collection and charcoal production (Church *et al*., 2007), one of the reasons for forest degradation, because livestock diets were, in addition to grassland forage, based on leaves, generative organs, bark and the annual twigs of woody species (Gauthier *et al*., 2010). In Iceland, Norse colonization followed by year-round livestock grazing and browsing has been considered one of the main reasons for the decline of *Betula pubescens* forests at the beginning of the 20th century (Ólafsdóttir and Guðmundsson 2002). Although woody species played, and in some Nordic regions still play, an important role in livestock feeding, information on the nutritive value of leaves (i.e. nitrogen, phosphorus and fibre fractions) of common woody species has been missing (see though Sigvaldason (1967) for earlier estimates). The aim of this study was to determine forage quality of leaves of common woody species (*Betula nana, B. pubescens, Salix lanata, S. phylicifolia* and *Sorbus aucuparia*) in Iceland and to compare it the with forage quality of two grasses, both common in old hayfields in Iceland: the native *Deschampsia cespitosa* and the high-yielding grass *Alopecurus pratensis,* introduced from Europe (Helgadóttir *et al*., 2013; Kristinsson, 2013).

Material and methods

We collected leaf biomass of five common broad-leaved woody species (*Betula nana, B. pubescens, Salix lanata, S. phylicifolia* and *Sorbus aucuparia*) and two grass species (*Deschampsia cespitosa* and *Alopecurus pratensis*) at four localities in Iceland in late June 2013. A total 28 biomass samples were oven-dried at 60 °C for 48 hours, ground to powder and analysed for the concentration of nitrogen (N), phosphorus (P) and the content of neutral- (NDF)

and acid-detergent fibre (ADF) and acid-detergent lignin. The N concentration in the plant samples was determined using an automated analyser TruSpec (LECO Corporation, USA) and P concentration using ICP–OES (Varian VistaPro, Mulgrave, Vic., Australia) after mineralization using *aqua regia* of burnt samples in in a microwave oven at a temperature of 550 °C. Contents of NDF, ADF and ADL were determined by standard methods of AOAC (1984). All analyses were performed in an accredited national laboratory, Ekolab Žamberk (http:// www.ekolab.zamberk.cz). Data were tested by one-way ANOVA followed by post-hoc comparison using the post-hoc HSD Tukey's tests to identify differences in concentrations of N, P, NDF, ADF and lignin contents among species.

Results and discussion

All values of concentrations of N, P, NDF, ADF and lignin are given in Table 1.

Table 1. Concentration (means ± standard error of mean) of N, P, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin in leaf biomass of studied species. Calculated by one-way ANOVA followed by Tukey post-hoc comparison test, significant differences (*P*<0.01) among species are indicated by different letters. Chemical properties of fodder from Icelandic grasslands (with dominant *Alopecurus pratensis*, *Poa pratensis*, *Phleum pratense* and *Agrostis capillaris*) follow Ragnarsson and Lindberg (2010) and Thorvaldsson *et al*. (1998) and optimum range for sheep and cattle follows Whitehead (1995).

Species	N (g kg⁻¹)	P (g kg⁻¹)	NDF (g kg⁻¹)	ADF (g kg⁻¹)	Lignin (g kg⁻¹)
Betula nana	24.1±1.0[a]	2.6±0.2 [a]	328±23 [ab]	305±15 [abc]	154±7 [d]
Betula pubescens	28.7±1.4 [a]	3.1±0.2 [ab]	315±16 [ab]	294±16 [abc]	123±10 [cd]
Salix lanata	27.5±0.9 [a]	3.8±0.4 [b]	376±25 [c]	345±22 [bd]	96±6 [bc]
Salix phylicifolia	27.7±2.2 [a]	3.9±0.3 [b]	272±28 [a]	243±15 [a]	119±19 [cd]
Sorbus aucuparia	26.5±1.3 [a]	3.0±0.3 [ab]	285±17 [ab]	269±12 [ab]	95±3 [bc]
Alopecurus pratensis	23.2±1.5 [a]	2.5±0.2 [a]	631±22 [d]	388±13 [d]	65±7 [ab]
Deschampsia caespitosa	25.1±0.8 [a]	2.0±0.2 [a]	614±12 [d]	324±6 [bcd]	38±2 [a]
Grassland forage optimum range for sheep/cattle	19-32	1.9-2.4	500-550	270-310	30-50
	19.2 - 25.6	2.3 - 3.7	330-450	190-300	max. 80

Concentrations of N were similar and concentrations of P, NDF, ADF and lignin were significantly different among species (Table 1). Nitrogen and phosphorus concentrations were also within the optimum range for nutrition of cattle in all analysed species, with the exception of the too-low P concentration in *D. cespitosa* and a slightly higher P concentration in *S. phylicifolia*. Optimum NDF content for sheep and cattle nutrition was recorded only in *S. lanata*, whereas optimum content of ADF was recorded in *B. pubescens*, *S. phylicifolia* and *S. aucuparia*. Content of lignin was substantially higher in woody species than in grasses. Relatively high lignin content in all woody species in comparison with grasses could be the most problematic for livestock metabolism, because digestibility of the biomass generally decreases with an increase in lignin content (Cherney *et al*., 1993). On the other hand, woody species offer considerable amounts of indispensable nutrients, particularly N and P. The nutritive value of leaves of the main woody species in Iceland was relatively high in comparison with the dominant broad-leaved woody species in Central Europe (Hejcmanová *et al*., 2013) and could satisfy livestock requirements. The forage quality of leaves of woody species increased in the order *B. nana* < *B. pubescens* < *S. phylicifolia* < *S. aucuparia* < *S. lanata*. Both *Betula* species are browsed by Icelandic sheep, but *B. pubescens* substantially more and mainly in the spring and early summer (Thorhallsdottir and Thorsteinsson, 1990) This pattern is in agreement with higher forage quality of *B. pubescens* than of *B. nana* and this probably explains why *B. pubescens* was harvested in the past for leaf fodder by Icelandic farmers while *B. nana* was rarely used (Gunnlaugsson, 1969). Very high P concentration was recorded in the leaves of both *Salix* species, probably due to lower temperatures and slower plant growth (Reich and

Oleksyn, 2004). We recorded that leaves of both *Salix* species were browsed by sheep in high quantity, much more than *B. nana* or *B. pubescens*, and that free-ranging sheep seem to be able to prevent regeneration of *Salix* shrubs in some Icelandic regions. Browsing of *Salix* species probably helps the animals to avoid P and Ca deficiency, especially high-milk-yielding ewes. *Salix* species are generally considered to be the best forage woody species in Nordic regions (Forbes *et al*., 2010; Myking *et al*., 2013).

Conclusions

The nutritive value of leaves of the main woody species in Iceland was relatively high and can satisfy livestock requirements for N and P. The most problematic for livestock metabolism would be the relatively high lignin content in all woody species, in comparison with grasses.

Acknowledgements

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Sensory quality and authentication of lamb meat produced from legume-rich forages

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Abstract

We investigated the dose-dependent response to dietary alfalfa:cockfoot proportion in grazing lambs of stable nitrogen isotope ratio in lamb meat and of fat skatole concentration and chop sensory attributes, and the ability of nitrogen isotope signature of the meat to authenticate meat produced from legume-rich diets. Four groups of nine male Romane lambs grazing a cocksfoot pasture were supplemented with different levels of fresh alfalfa forage to obtain four dietary proportions of alfalfa (0%, 25%, 50% and 75%) for 98 days on average before slaughter (groups U, L, M and H). The distribution of the δ^{15}N values of the meat discriminated the U lambs from the H lambs, and gave a correct classification score of 85.3% comparing lambs that ate alfalfa with those that did not. Perirenal fat skatole concentration decreased linearly with the δ^{15}N value of the muscle, indicating a linear increase with the dietary proportion of alfalfa. The intensity of 'animal' flavour increased from the lowest level of alfalfa onwards and did not increase further with increasing levels of alfalfa, suggesting that this sensory attribute reaches a plateau when perirenal fat skatole concentration is in the range 0.26–0.34 μg/g fat.

Keywords: authentication, flavour, grazing, legumes, scatole, sheep

Introduction

Low-input and organic farming livestock systems embody features that consumers value, such as animal welfare, food healthiness and environmental acceptability. The ability to authenticate food products from these agro-ecological systems has therefore become an important challenge. Nitrogen (N) isotope signature has been proposed as a valuable tool to authenticate meat produced from organic beef (Bahar *et al.*, 2008). The presence of forage legumes in organic systems is actually of major importance, because nitrogen-fixing plants improve pasture quality and reduce dependency on external inputs, and the dietary ^{15}N/^{14}N ratio is lower in legume-rich pastures due to the capacity of leguminous plants to fix atmospheric nitrogen. However, the occurrence of off-flavours and off-odours in the meat has been shown to increase in lambs grazing legume-rich pastures (Young *et al.*, 2003). Flavours in lamb meat described as animal, pastoral and faecal have been related to the presence of skatole, which is an aromatic compound produced in rumen from the tryptophan amino acid. Its ruminal synthesis increases when the pasture is rich in forage species with high degradable protein content. This study therefore investigated i) the dose-dependent response to dietary alfalfa levels in grazing lambs of stable nitrogen isotope ratio in lamb meat and of fat skatole concentration and chop sensory attributes, and ii) the ability of nitrogen isotope signature of the meat to authenticate meat produced from legume-rich diets.

Materials and methods

Four groups of nine male Romane lambs grazing a cocksfoot pasture were supplemented with fresh alfalfa forage to obtain four dietary proportions of alfalfa (0%, 25%, 50% and 75%) for 98 days before (groups U, L, M and H). The mean daily voluntary dry matter intake was

estimated for each group at 78.1 g DM/BW$^{0.75}$. Alfalfa was cut every morning and offered half at 9 a.m., and half at 4 p.m. after storage at 4 °C. We measured the ^{15}N/^{14}N ratio in the forages and in the *longissimus thoracis et lumborum* (LTL) muscle by isotope ratio mass spectrometry. We analyzed the data using variance analysis (GLM procedure of SAS) and linear discriminant analysis followed by a cross-validation procedure to classify the meat samples according to feeding treatments, using Minitab software v.13. Perirenal fat skatole concentration was measured by HPLC. Lamb chop flavour and odour was evaluated by experienced panellists. Six chops per lamb were grilled to an internal temperature of 75 °C and served to 12 panelists. Two pieces from the lean part and two pieces from the fat part were cut from each chop to provide a piece of each for each panellist. At each session, the panellists evaluated one lamb per treatment, with lambs from each treatment presented in randomized order. A preliminary session was performed using additional chops from one lamb with the highest skatole concentration and one lamb with the lowest skatole concentration, for the panellists to agree on common terms describing the perceptions related to the presence of skatole. These were 'animal' flavour and 'animal' odour. The data for lamb chop sensory evaluation underwent an ANOVA analysis using a mixed model, with treatment and panel session as fixed factors and panellist as a random factor, and using the Bonferroni test for pairwise comparisons. Full details are given in Devincenzi *et al.* (2014a and b).

Results and discussion

Mean daily alfalfa intake was 0, 272, 550 and 731 g DM, which corresponded to a mean dietary proportion of alfalfa of 25.8%, 48.7% and 62.4% for L, M and H lambs respectively. The δ^{15}N value of the LTL muscle decreased linearly with the dietary proportion of alfalfa (PA, %) ($P = 0.024$), the regression equation being: δ^{15}N value of the LTL muscle = 6.04 ($\pm$0.071) − 0.0107 ($\pm$0.00169) PA, where $r^2 = 0.95$, RSD = 0.080, and $n = 4$. The distribution of the δ^{15}N values of the meat discriminated all the U lambs from the H lambs, and gave a correct classification score of 85.3% comparing lambs that ate alfalfa with those that did not.

Perirenal fat skatole concentration was higher for L and M lambs than for U lambs (Figure 1A). Surprisingly, perirenal fat skatole concentration in H lambs was lower than that of M lambs, and it was not statistically different from that of U lambs. As this result may be partly linked to the variability between individual animals in dietary choices, we used the δ^{15}N value of the LTL muscle as an indicator of the dietary proportion of alfalfa at the individual level and explore the dose-dependent response further. Perirenal fat skatole concentration decreased linearly with δ^{15}N value of the LTL muscle ($P < 0.03$, Figure 1C), supporting the hypothesis that the fat skatole concentration increases linearly with the dietary proportion of alfalfa.

The intensity of 'animal' odour in the lean part of the chop and of 'animal' flavour in both the lean and fat parts of the chop increased from the lowest level of alfalfa supplementation onwards and did not increase further with increasing levels of alfalfa supplementation (Figure 1B). The outcome of this study therefore suggests that these sensory attributes may reach a plateau when perirenal fat skatole concentration is in the range 0.16-0.24 µg/g of liquid fat.
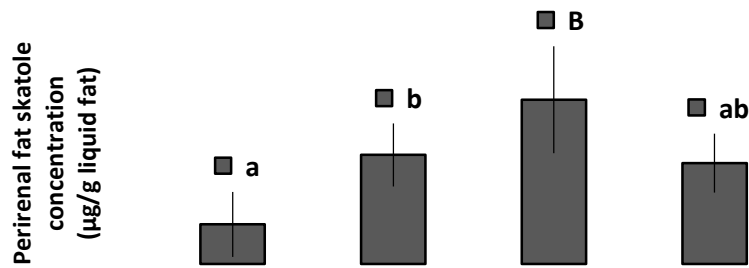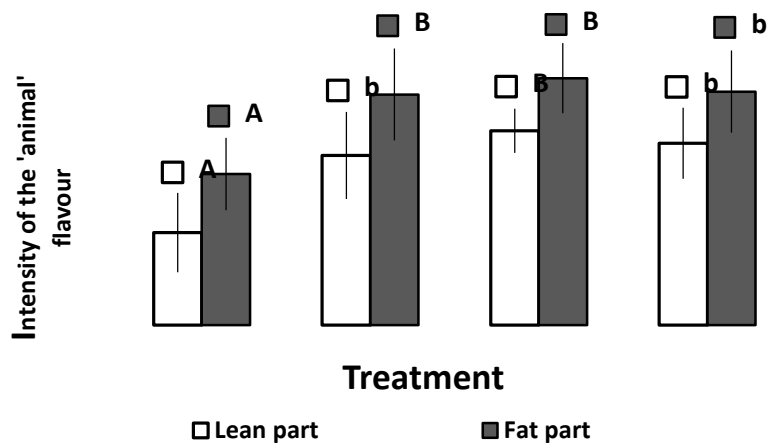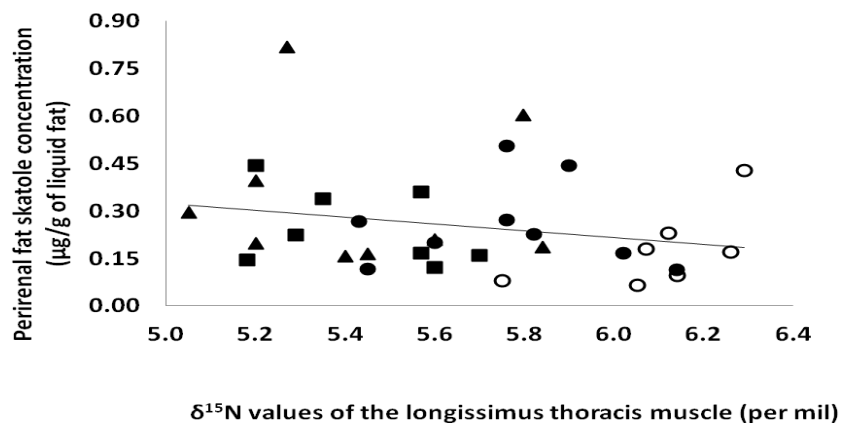
Figure 1: (A) Mean value of perirenal fat skatole concentration according the dietary level of alfalfa in grazing lambs. Means with unlike superscripts differ (A, b: $P < 0.05$; a, b: $P < 0.07$). (B) Mean intensity of 'animal' flavour in the lean and fat parts of the chops according the dietary level of alfalfa in grazing lambs. For the lean and the fat parts, means with unlike superscripts differ (A, b: $P < 0.05$; A, B: $P < 0.01$). Bars represent standard error of the mean (SD/√n), where n is the number of lambs in each group. (C) Relationship between perirenal fat skatole concentration and $\delta^{15}N$ value of the *longissimus thoracis et lumborum* muscle. White circles refer to un-supplemented lambs, black circles, triangles and squares refer to groups of lambs receiving a low, medium or high level of alfalfa supplementation respectively.

Conclusions

Perirenal fat skatole concentration was higher for lambs that consumed alfalfa than for those that consumed only cocksfoot, and it increased as soon as the dietary proportion of alfalfa reached 25%. The intensity of 'animal' odour in the lean part of the chop and of 'animal' flavour in both the lean and fat parts of the chop were increased from the lowest level of alfalfa supplementation onwards and did not increase further with increasing levels of alfalfa supplementation. The outcome of this study therefore suggests that these sensory attributes may reach a plateau when perirenal fat skatole concentration is in the range 0.16-0.24 µg/g of liquid fat. The distribution of the $\delta^{15}N$ values of the LTL muscle discriminated all the U lambs from the H lambs, and gave a correct classification score of 85.3% comparing lambs that ate alfalfa with those that did not. These results may be of interest for the authentication of meat produced in low-input and organic production systems, in which leguminous plants are more widespread.

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Dynamics of dry matter intake in livestock production systems in the Netherlands

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Abstract

Pasture-based dairy systems have several advantages. However, the number of grazing dairy cattle in the Netherlands is decreasing, partly as a result of scaling. Therefore, grazing research focuses on providing farmers, students and advisers with tools to optimize grazing whilst also improving efficiency. The aim of this study was i) to estimate the dry matter intake (DMI) from grazing on commercial dairy farms and the variation of this DMI throughout the season, ii) to estimate the associated feeding costs, and iii) to present this in a practical way. A DMI-dashboard was developed to get day-to-day insight in DMI and feeding costs. It was tested on nine commercial dairy farms. Data show that there was a huge difference in DMI by grazing (800 to 1900 kg DM cow^{-1} yr^{-1}). The between-farm variation in feeding costs was much larger than the within-year variation and is a good reflection of the differences in DMI by grazing between the different individual farms. By comparing results of individual farms in a network setting, the insight in the effect of grazing increased. This led to increased skills of farmers, students and advisers in optimizing grazing systems.

Keywords: Dry matter intake (DMI), feeding costs, grazing, pasture, skills

Introduction

The number of grazing dairy cattle in the Netherlands has decreased in recent years, from 90% the total in in 2001 to 70% in 2012 (CBS, 2013). In 2012, the 'Convenant Weidegang' ('Treaty Grazing') was therefore initiated. The aim of the Treaty is to stabilize the percentage of farms that practise grazing. At the end of 2013 almost 60 parties in the Netherlands had signed this Treaty, including dairy farmers, dairy industry, feed industry, banks, accountant, semen industry, veterinarians, cheese sellers, retail, NGOs, nature conservation, government, education and science. As a result of the Treaty, many research activities have been initiated to optimize grazing and grassland management. In the last few years, the theme 'grazing' has also become more dominant in education. The interaction between education and science increasingly leads to participation of lecturers and students in scientific research on grazing, which in turn leads to an improved educational programme.

Grazing is, on average, economically attractive (Van den Pol-van Dasselaar, 2014) and is preferred by society. However, dry matter yields are lower, nutrient losses are higher and often farmers experience grazing as difficult. This is especially true in the Netherlands with its mixed dairy systems that use relatively high levels of supplementation. Most farmers and advisers lack the necessary skills to manage grazing properly in such high-output systems. Support is needed and this support should be robust, simple and appealing (Van den Pol-van Dasselaar *et al.*, 2012).

The focus of grazing research in the Netherlands is to provide farmers, students and advisers with the tools to optimize grazing under Dutch conditions whilst also improving efficiency. The aim of this study was i) to estimate the dry matter intake (DMI) from grazing on commercial dairy farms and the variation of this DMI throughout the season, ii) to estimate the associated feeding costs, and iii) to present this in a practical way. Currently, these data are not available

on commercial dairy farms in the Netherlands. And if one does not measure, one cannot manage.

Materials and methods

In 2012 and 2013, participatory research on nine commercial dairy farms of the network 'Dynamisch Weiden' (Dynamic Grazing) was carried out to study the dynamics of grazing and to develop practical management tools. Researchers, advisers and dairy farmers together searched for suitable grazing systems. Farmers were encouraged to optimize their grazing system and to gain new knowledge and skills. The work was supported by students. The farms differed in scale, soil type, grazing intensity (cows ha^{-1}, hours day^{-1}, days on one paddock). Grazing and supplemental feeding was recorded in a grassland calendar. A DMI-dashboard was developed to get insight in day-to-day DMI and feeding costs. Total feed demand was estimated using characteristics of the herd, like number of cows, milk yield per cow, fat and protein content of the milk. The grass intake was calculated by subtracting the supplemental feed from the total feed demand. The feeding costs were calculated using the actual feed costs for supplemental feeding and estimated feed costs for home-grown forage.

Results and discussion

The dairy farmers in the network were eager to get insight in the results. They confirmed that for them the difficulty of grazing is the variation in milk production due to the unknown variation in grass growth, grass quality and DMI. A tool to get insight in more accurate grass intake was therefore considered to be very useful. An example of such a tool is the DMI-dashboard, which has been developed in the project (Figure 1).
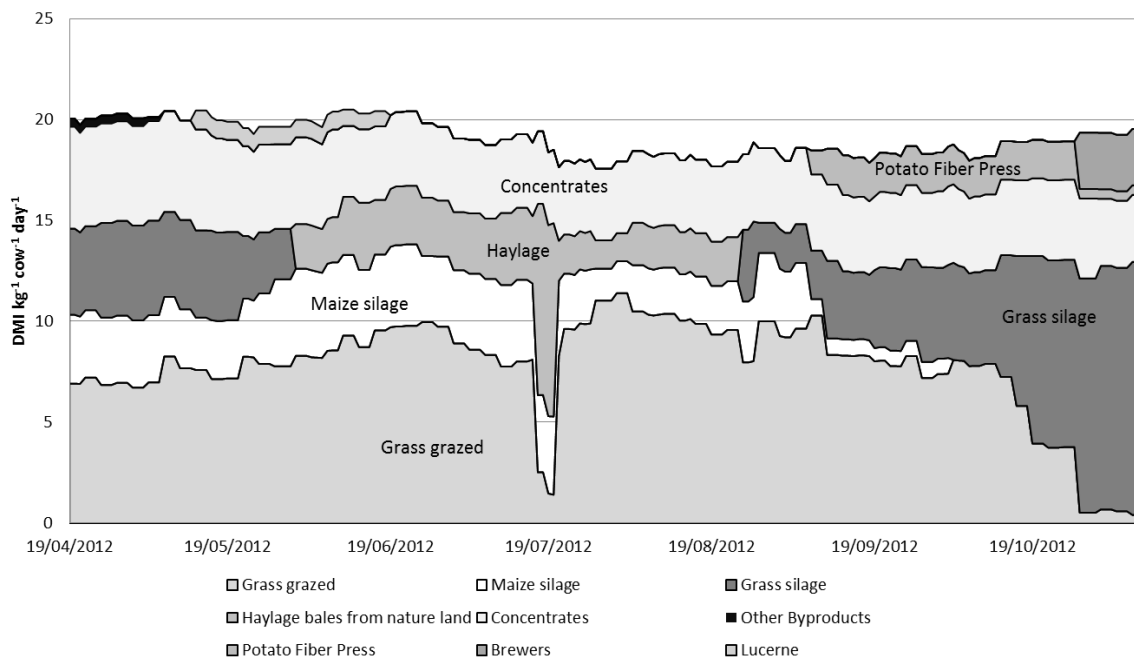
Figure 1. The DMI-dashboard for an individual farm in the growing season 2012.

The DMI-dashboards of the nine commercial dairy farms showed that the DMI from grazing varied between 800 and 1900 kg DM cow^{-1} yr^{-1}. A recent Dutch study into the economics of grazing has shown that grazing is financially attractive if the cows eat sufficient amounts of fresh pasture grass (> 600 kg DM cow^{-1} yr^{-1}). If the intake of fresh grass is very low, grazing is not profitable (Van den Pol-van Dasselaar *et al*., 2014). The feeding costs were determined for the nine farms throughout the season. Figure 2 shows that the between-farm variation in feeding costs was much larger than the within-year variation. The between-farm variation

reflects the differences in DMI by grazing between the different individual farms. The dairy farmers in the study experienced these results as extremely valuable even though the costs of some individual feed components were only estimated. The within-year variation and the between-farm variation provided an opportunity for them to benchmark their individual farm results, which contributed to overall improvement of the grazing system. The effect of management options, e.g. ration changes, was immediately visible and operational management could be adjusted accordingly.
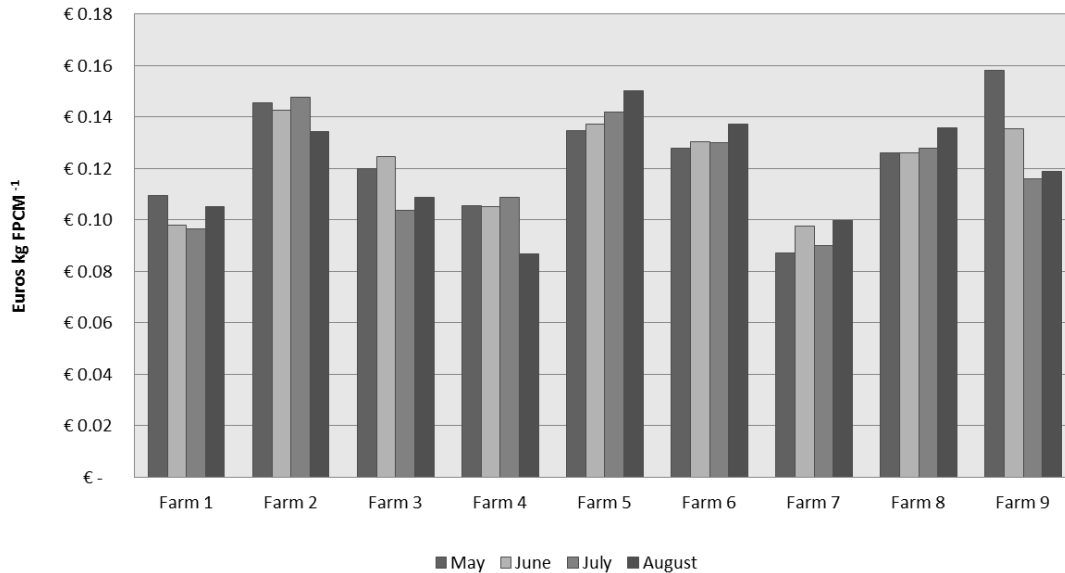
Figure 2. Feeding costs in May, June, July and August 2013 for nine commercial dairy farms.

Conclusion

Today, many farmers in the Netherlands do not focus on grassland in their operational farm management. By comparing results of individual farms in a network setting, the insight in the effect of grazing increased. This has led to increased skills of farmers, students and advisers in optimizing grazing systems.

Acknowledgements

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Cutting strategy of a five-cut system in different grassland mixtures

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Abstract

A stronger focus on the nutritive value throughout the growing season is needed to improve the utilization of annual grass-clover production. A field experiment tested five different cutting strategies with a total of five cuts per year and with different timings of the spring cut and duration of regrowth in three mixtures. The cutting strategies highly influenced seasonal growth and nutritive value and only slightly influenced the annual dry matter yield. Delaying the spring cut decreased the annual IVOMD and percentage of crude protein, whereas the annual percentage of NDF increased. Thus, the higher nutritive value in the shorter regrowth periods later in the season could not make up for the lower value in the late spring cut. The annual white clover content decreased and red clover content increased with a later spring cut. The results indicate that especially with mixtures containing red clover a planned cutting strategy is necessary.

Keywords: cutting strategy, red clover, white clover, nutritive value, digestibility

Introduction

There is often a keen focus on optimizing the nutritive value of the spring cut and less focus on the rest of the growing season. However, the complete herbage production of the year is used on the farm for feeding different cattle groups with different requirements. The challenge is to optimize the nutritive value of that part of the seasonal production which is planned for feeding to high-yielding dairy cows. Herbage cuts are often co-ensiled in horizontal silos, which is one of the reasons why a planning tool for timing the cutting time, and which also indicates which cuts should be co-ensiled, could be useful. By comparing different feeding trials, Weisbjerg *et al*. (2011) showed that milk yield did not increase further when *in vitro* organic matter digestibility (IVOMD) of the grass-clover part of the feeding ration increased above 78%, and that the point was independent of stage in the growing season. This means that IVOMD can be used as a goal when planning the seasonal cutting strategy. Here results are presented from an experiment with different cutting times and durations of regrowth with a focus on the yearly mean feeding quality.

Materials and methods

Five different harvest strategies with five cuts per year (Figure 1) were tested on a sandy loam at Foulum in Denmark in three mixtures (26 kg seed ha^{-1}), made up of either (1) 13% white clover (*Trifolium repens* L.) and 87% perennial ryegrass (*Lolium perenne* L.), (2) 11% red clover (*Trifolium pratense* L.), 7% white clover, 37% perennial ryegrass and 45% festulolium (*Festulolium braunii K.A.*) or (3) pure red clover only, cultivated for strategies 2 and 4. The swards were fertilized with 100 kg N ha^{-1} in spring and 60 kg N ha^{-1} after the second cut. There were four replicates. The grass-clover was undersown in spring barley (*Hordeum vulgare*) in 2011. Plots were harvested with a Haldrup plot harvester, nutritive value was determined by NIR, IVOMD calibrated to the method of Tilley and Terry, and botanical composition of dry matter (DM) was determined by hand separation of subsamples.

Strat.	May	June	July	August	September	Oct.
1	a)	5	5	5		6
2	b)	4	4	6		6
3	b)	5	5	4		6
4	c)	5	5	4		5
5	d)	5	4	5		4

Figure 1. Harvest strategy. Date of spring cut and length of regrowth period in number of weeks.
Date for spring cut in 2012/2013: a) 14/23 May, b) 22/28 May, c) 29 May/4 June and, d) 6/11 June.

Results and discussion

The two harvest years differed weather-wise. The 2013-season was warmer and drier than 2012. However, the principal differences were comparable. Harvest time strongly influenced the seasonal profile of dry matter production and quality parameters (Figure 2). Early harvest in spring gave a more even seasonal production profile and the IVOMD was highly affected by both time of spring harvest and duration of regrowth. The strategies were planned to reach different goals. Strategy 3 aimed for an even nutritive value throughout the season, and therefore the third regrowth in late summer was short due to the normally higher temperature in this period, which gives a lower digestibility of organic matter. In contrast, in strategy 2 the third regrowth period was long and the durations of regrowth 1 and 2 were short, with the goal of optimizing the nutritive value before the warm period and then to produce herbage with a lower nutritive value in the warm period. For strategy 3 the goal was only partly reached, as IVOMD in the second regrowth was relatively low. Harvest time for this 5-week regrowth period was thus too late in the summer.
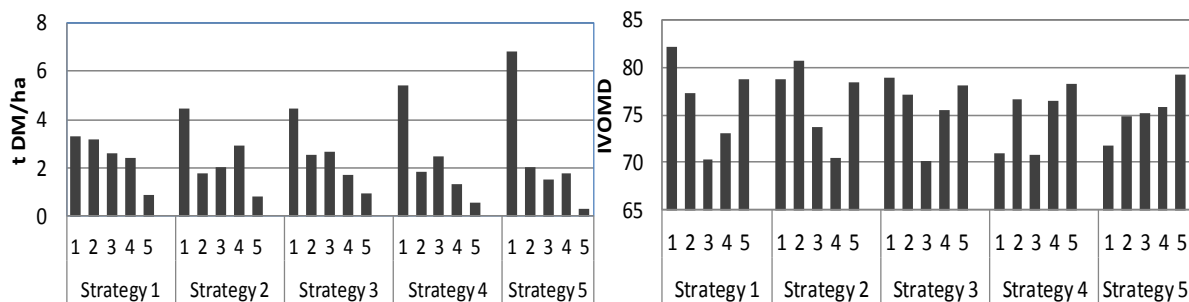
Figure 2. Dry matter yield (left) and digestibility of organic matter (right) per cut in mixture 2 (perennial ryegrass, festulolium, white and red clover). Mean of 2012 and 2013.

Despite the very different cutting strategies, the annual yields were very similar (Table 1). The exception was strategy 4, with a lower annual yield due to poor growth in the last week before spring cut in both years. Including red clover and festulolium in mixture 2 increased the annual yield by an average of only 0.9 t DM ha^{-1}. IVOMD was lower in mixture 2 than in mixture 1 and lowest in pure red clover (mixture 3). Red clover often has a lower IVOMD than grass (Kuoppala, 2010). Since red clover constituted a third of the herbage in mixture 2 (Table 1), this was the main reason for its lower IVOMD. Crude protein and NDF contents were nearly the same in mixtures 1 and 2. The annual content of white clover decreased with the later spring cut in mixtures 1 and 2, whereas the content of red clover in mixture 2 increased.

In general, the yearly nutritive value decreased with a later spring cut. The yearly IVOMD and crude protein content decreased whilst NDF content increased. The percentage of annual yield harvested with an IVOMD higher than 78 is shown in Table 1. Late harvest of the spring cut decreased this proportion with a high IVOMD. Especially in mixture 2 was this significant, as

only a small part, 9-14%, had a high IVOMD with a late spring harvest. In pure red clover (mixture 3) only the autumn cut had a high IVOMD. The results indicate that the effect of cutting strategy is greater in mixtures with red clover.

Under comparable conditions, the nutritive value is normally higher in the spring cut than later in the season. Our theory was that a later spring growth, which means a high yield in the period when the quality is potentially higher, together with an adapted cutting strategy for the rest of the growing season, which means a lower yield in the period with lower quality, could give a higher mean herbage quality overall. However, the results showed the opposite.

Table 1. The annual dry matter yield and weighted mean of white and red clover content, IVOMD, crude protein and NDF, and the percentage of the harvested herbage with an IVOMD higher than 78.

Mixture	Strategy	Yield (t DM/ha)	Clover content (% of DM) White cl.	Red cl.	IVOMD (% of OM)	Crude protein (g/1000 g DM)	NDF	IVOMD %>78
1	1	11.3[a]	37.2[a]		78.7[a]	190[a]	397[b]	61
	2	11.6[a]	36.6[a]		79.0[a]	196[a]	381[c]	54
	3	11.3[a]	35.8[a]		79.3[a]	189[a]	386[bc]	76
	4	10.4[b]	29.8[b]		76.5[b]	184[a]	426[a]	43
	5	11.6[a]	27.4[b]		77.2[b]	159[b]	434[a]	45
	Lsd	0.4	5.7		1.3	14	12	
2	1	12.5[a]	19.3[a]	31.8[c]	76.7[a]	200[a]	384[a]	44
	2	12.[ab]	18.7[a]	34.1[bc]	76.2[a]	199[a]	378[a]	48
	3	12.3[a]	18.6[a]	32.9[bc]	76.3[a]	195[a]	384[a]	50
	4	11.7[b]	12.7[b]	38.9[a]	72.9[b]	185[b]	432[a]	14
	5	12.5[a]	10.4[b]	35.1[b]	73.6[b]	167[c]	421[a]	9
	Lsd	0.7	3.5	3.2	1.2	10	12	
3	2	11.1			74.2[a]	224	316[b]	8
	4	10.2			71.0[b]	219	357[a]	2
	Lsd	ns			3.0	ns	24	

Different superscripts within mixtures indicate a significant difference (*P<0.05*)

Conclusion

The results showed the potential to influence the nutritive value with minimal effect on the dry matter production. Especially for mixtures containing red clover, the strategy seemed to be essential for the yearly mean of nutritive value. The seasonal profile of nutritive value and production was, in general, strongly affected by the cutting strategy, and the results indicate opportunities for designing herbage quality to meet different end-purposes. To optimize the herbage production for its planned use on the farm, a decision support system is needed. To achieve this, well-defined goals of nutritive value dependent on the proportion of clover in the herbage are necessary.

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Theme 4 posters

Conserving high moisture spring field bean (*Vicia faba* L.) grains

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Abstract

The high protein content of bean (*Vicia faba* L.) grains give them the potential to be used as a home-grown feedstuff that could replace soyabeans in ruminant diets. Crimping and additive treatment of beans, which were harvested at a high moisture content and then ensiled in laboratory silos, were assessed for their effects on aspects of conservation efficiency. Whole or crimped bean grains (751 g dry matter kg^{-1}) were ensiled for 160 d either without additive or following the application of acid, urea, *Lactobacillus buchneri* or *Lactobacillus plantarum* plus *Pediococcus pentosaceus*-based additives. The grains in all treatments conserved successfully, undergoing limited fermentation and in-silo losses, and being relatively stable during feedout. Crimping stimulated a more extensive fermentation but increased in-silo losses and the susceptibility to aerobic losses at feedout. Each additive had its unique influence with no single additive improving all traits.

Keywords: *Vicia faba*, ensile, crimp, additive, chemical composition, losses

Introduction

Field beans (*Vicia faba* L.; also known as faba, broad, tick or horse bean) are pulse legumes whose grains are included in cattle rations mainly for their high protein content, but whose available energy content is at least as good as cereal grains (McDonald *et al*., 2011). They are normally stored dry and, prior to feeding to ruminants, they are processed by cracking, rolling, coarse-grinding or steam-flaking. Grains with a moisture content higher than optimal for extended storage can be aerobically stored following artificial drying or treatment with agents such as propionic acid or ammonia that inhibit aerobic microbial activity. An option for beans of higher moisture content is to ensile them with or without physical processing pre-ensiling, and the opportunity exists to manipulate the ensilage process by treating the harvested grains with additives that restrict or enhance fermentation. These options have previously been explored for high-moisture barley grains (Stacey *et al*., 2011). This experiment assessed the effects of crimping and additive treatment of bean grains, harvested at a high moisture content and then ensiled, on their subsequent chemical composition, in-silo loss and aerobic stability characteristics.

Materials and methods

Spring field bean (cv. Scirocco) seed was sown at 150 kg ha^{-1} on 15 February at Oak Park in Carlow, Ireland, and received 185 kg of 0-7-30 fertilizer ha^{-1}. It was combine-harvested on 2 September and representative samples were treated with the following additive treatments: (1) no additive (NA), (2) Graintona (FSL Bells Ltd., UK; acetic acid, isobutyric acid mixture) at 8 l t^{-1} (Acid), (3) NuGrain (Hydro Nutrition, Hydro Agri (UK) Ltd.; urea solution) at 50 l t^{-1} (Urea), (4) Biograin (Biotal Ltd., Wales; *Lactobacillus buchneri*) at 10 l t^{-1} (B1), and (5) Siloking (Agri-King, Inc., USA; *Lactobacillus plantarum*, *Pediococcus pentosaceus*) at 400 g t^{-1} (B2). For the B1 treatment, the DM concentration of grain was quickly assessed by microwave drying. The whole or rolled grain was then placed in a water-tight mixer with a quantity of water sufficient to reduce grain DM concentration to 550 g kg^{-1}, and continuously mixed for up to 15 minutes. After removing unabsorbed water, the additive was applied at 8 l t^{-1}. The B2 was applied as a dry formulation. All additives were intimately mixed with the grains

prior to ensiling. Approximately 4 kg grain dry matter (DM) were ensiled in each of triplicate laboratory silos per treatment, without compression within each silo. Silos were stored for 160 days at approximately 15°C. In-silo DM loss values were calculated from the change in weight of herbage DM between the start and end of ensilage (both dried at 98°C for 16 h). A sub-sample of each silage was subsequently stored aerobically in an insulated container at 20°C for 192 h, and the duration until its temperature rose >2°C above a reference ambient value (aerobic stability) and the accumulated temperature rise during the first 120 h exposure to air (aerobic deterioration) were calculated. Chemical composition, in-silo loss and aerobic stability data were statistically analysed using a general linear model that accounted for crimping, additive and the crimping x additive interaction.

Results and discussion

A grain fresh yield of 4886 kg ha^{-1} was recorded with a mean (s.d.) composition at ensiling of DM 751 (7.0) g kg^{-1}, *in vitro* DM digestibility (DMD) 804 (9.1) g kg^{-1}, ash 35 (0.3) g (kg DM) $^{-1}$, starch 335 (5.5) g (kg DM) $^{-1}$, water-soluble carbohydrates 130 (4.4) g (kg DM) $^{-1}$, crude protein 255 (2.1) g (kg DM) $^{-1}$ and buffering capacity 209 (1.9) mEq (kg DM) $^{-1}$. The high-moisture spring field-bean grains underwent relatively minor quantitative or qualitative losses during ensilage, and therefore conserved successfully. Crimping increased post-ensilage starch, lactic acid, acetic acid, lactic acid as a proportion of fermentation products (LA/FP), NH$_3$-N, in-silo loss ($P<0.001$) and aerobic deterioration ($P<0.05$) values; it reduced DM, pH ($P<0.001$) and aerobic stability ($P<0.01$) values, and it had no effect on DM digestibility (DMD), ash, crude protein, ethanol and WSC values ($P>0.05$) (Table 1).

Table 1. Spring field bean grain silage composition, recovery rate, and aerobic stability and deterioration characteristics

Crimp (C)	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes		*P* value		
Additive (A)	NA	Acid	Urea	B1	B2	NA	Acid	Urea	B1	B2	SEM[1]	C	A	C x A
DM[2]	734	731	718	699	741	729	721	694	687	722	4.1	.188	<.001	<.001
DMD[2]	822	792	817	795	802	813	805	809	794	805	8.4	.720	.970	.061
Ash[3]	36	35	34	36	36	36	36	35	37	36	0.4	.866	.094	<.001
Starch[3]	328	308	297	318	315	340	323	319	343	340	7.0	.833	<.001	.007
C. protein[3]	291	288	336	295	286	289	286	328	295	288	2.5	.316	.226	<.001
pH	6.1	5.9	8.8	5.6	6.1	5.9	5.9	8.9	4.9	5.2	0.05	<.001	<.001	<.001
Lactic acid[3]	2	2	6	13	4	7	5	5	23	15	0.6	<.001	<.001	<.001
Acetic acid[3]	1	2	4	3	1	2	2	4	8	3	0.3	<.001	<.001	<.001
Ethanol[3]	5	7	3	11	7	8	4	3	9	5	1.0	.075	.362	<.001
Lactic/FP[4,2]	281	203	480	478	341	394	414	426	582	665	50.6	.018	<.001	.001
WSC[3]	86	72	88	63	84	75	94	81	60	69	3.2	.189	<.001	<.001
NH$_3$-N[5]	2	2	26	7	2	3	3	24	11	5	0.4	<.001	<.001	<.001
In-silo loss[6]	32	36	45	71	23	38	44	101	98	56	11.1	.001	<.001	.197
Aer.stab.[7]	5	10	10	9	5	4	3	7	10	3	1.5	.147	.009	.003
Aer. det.[8]	7	1	3	3	10	13	11	5	2	12	2.8	.327	.040	.026

[1]For C x A interaction; [2]g kg^{-1}; [3]g (kg DM)$^{-1}$; [4]Fermentation products (lactic+acetic+propionic+butyric acids, and ethanol); [5]g (kg N)$^{-1}$; [6]g DM (kg DM)$^{-1}$; [7]Days; [8] °C

Propionic and butyric acids were at low concentrations or absent. Beans conserved without an additive underwent a restricted fermentation (12.9 g FP (kg DM)$^{-1}$) with little proteolysis, and exhibited low in-silo losses. The Acid additive reduced pH without altering the extent or direction of fermentation. It caused a small decline in both starch and DMD values. The Urea treatment increased NH$_3$-N, crude protein, acetic acid, pH and in-silo loss values, and reduced both starch and DM values. Both B1 and B2 reduced pH by increasing both lactic acid and LA/FP values, even though they both also increased NH$_3$-N values. B1 additionally reduced DM, DMD and aerobic deterioration, and increased acetic acid, ethanol, in-silo losses and aerobic stability. B1 had a larger effect on pH, lactic acid and NH$_3$-N values than B2. Crimping x additive interactions occurred, with crimping eliciting a larger pH decline in response to B1 and B2, and a smaller pH decline for Acid and Urea, than occurred for the NA treatment ($P<0.05$). This reflected a larger increase in lactic acid concentration in response to crimping for B1 and B2 than for the other treatments ($P<0.05$). The *Lactobacillus buchneri* in B1 increased the concentration of acetic acid, an effect most evident with crimped grain. Overall, these results with high-moisture bean grains agree with the findings of Stacey *et al*. (2011) and Stacey *et al*. (2005) relating to high moisture barley and wheat grains, respectively.

Conclusions

High-moisture spring field-bean grains were successfully conserved by ensilage, undergoing a limited fermentation and in-silo loss, and being relatively stable during aerobic feedout conditions. Under the circumstances of the evaluation, crimping stimulated a more extensive fermentation but increased in-silo losses and susceptibility to aerobic losses at feedout. Each additive had its unique influence on conservation characteristics and nutritive value, with no single additive being successful in its influence on all traits. Caution is required if extrapolating these results to what might happen on farms due to the greater challenges associated with rapidly achieving and maintaining anaerobiosis in farm silos.

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Fava bean-rapeseed intercrop as a sustainable alternative to Italian ryegrass: production, forage quality and soil fertility evolution

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Abstract

The maize-Italian ryegrass forage rotation is very common among dairy farms due to its high productivity. However, this rotation is very demanding in terms of nitrogen fertilization, with negative effects on soil fertility. This has led the search for new and more sustainable forage sources, leading to a renewed interest in using cattle manure and slurry application to grow forage crops. Under the premise of keeping the maize as summer crop, an alternative winter intercrop (fava bean and rapeseed) to the Italian ryegrass was evaluated, in order to combine the N-fixing abilities of legumes with the ability of cruciferous species to mobilize soil nutrients. The results showed that the fava bean-rapeseed intercrop may be a sustainable alternative to Italian ryegrass as a winter crop, because there are differences in protein and energy performance between these forages. Furthermore, it use allows the reduction of inputs of synthetic fertilizers and herbicides, and has a positive effect on the balance of soil nutrients, especially by increasing the potassium content and maintaining the soil pH.

Keywords: sustainability, forage, nutritive quality, organic fertilization, soil fertility

Introduction

The efficiency of chemical fertilizers used in maize cropping has become a major issue of concern, as the crop is often negatively associated with N-losses and impacts on surface and groundwater quality (Schröder *et al.* 2000). External inputs of nitrogen and phosphorus on farms should be reduced for environmental and economic reasons. As an alternative to chemical fertilizers, manure application to crop fields can recycle animal wastes and be a valuable soil nutrient resource. A number of studies have reported the on the benefits application of dairy manure on maize silage production (Butler *et al.* 2009). Furthermore, the production of forages today must be environmentally and ecologically sound, and aligned with public values. Fava beans (*Vicia faba*) through their fixing of atmospheric nitrogen, with high production, high protein content, and highly digestible and acceptable ensilability, are attractive for sustainable forage production (Martínez-Fernández *et al.*, 2010). Rapeseed has a powerful and deep root system that mobilizes nutrients from deeper layers to the surface and has a 'herbicidal action' by reducing weed growth (Grundy *et al.*, 1999) and maintaining soil fertility (Liebman and Davis, 2000). The aim of this study was to use a fava bean and rapeseed intercrop as an alternative to Italian ryegrass as a winter crop, in combination with the utilization of organic fertilization versus conventional management. The results of two consecutive agronomic years (Sept 2011-Sept 2013) are reported in this study.

Materials and methods

Two adjacent plots of 1.7 ha each were managed with either standard (SA) or alternative approach (AA). The annual fertilization of SA plot was with a basal fertilizer dressing of 60 kg N ha^{-1}, 40 kg P$_2$O$_5$ ha^{-1} and 120 kg K$_2$O ha^{-1} before sowing the winter crop (*Lolium multiflorum* Lam.), 70 kg N ha^{-1} applied as topdressing after the first spring cut for silage, and 125 kg N ha^{-1}, 144 kg P$_2$O$_5$ ha^{-1} and 216 kg K$_2$O ha^{-1} after second silage cut, before sowing the maize. When maize plants were 20 cm high, 75 kg N ha^{-1} was applied as a topdressing. The AA plot was fertilized with 32 m^3 ha^{-1} of slurry and 36 t ha^{-1} of manure before sowing the associated winter

crop. Before the maize sowing, 33 t ha^{-1} of manure and 84 m^3 ha^{-1} of slurry were applied. For the winter crops, the AA plot was sown with a fava bean-rapeseed intercrop (*Vicia faba* cv. Prothabon with *Brassica napus* cv. Fricola; HC) at seed rates of 150 and 8 kg ha^{-1} respectively. The SA plot was sown with 45 kg ha^{-1} of Italian ryegrass (*Lolium multiflorum* cv. Barextra; RI). As the summer crop, maize (*Zea mays* cv. Crazy) was sown in both plots, with a seeding density of approximately 90 000 plants ha^{-1}. For weed control in maize, a selective herbicide was used in SA management at a dose of 4 L ha^{-1} at sowing. In AA management, the herbicide dose was reduced by half to evaluate the effect of weed control by the rapeseed. Before each harvest, samplings for yield and quality of the fresh forage were made. Fresh forage samples were dried at 60ºC for 24h for dry matter analysis (DM), then ground (0.75 mm) and analysed by NIRS for organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), organic matter digestibility and metabolizable energy. To evaluate the change in the soil profile by both managements, taking as reference the composition thereof in May 2011, the soil was sampled in both plots between harvests. Statistical analysis was performed as factorial model, with the management and year as main factors (SAS, 1999). The evolution of soil characteristics was evaluated by a model for repeated measures.

Results and discussion

Table 1 shows the forage production of two consecutives agronomic years in both managements for winter and summer crops.

Table 1. Forage production (t DM ha^{-1}) under two different managements in 2012 and 2013.

	Standard		Alternative		sem	$P=$		
	2012	2013	2012	2013		Management	Year	Interaction
Winter crop[1]	7.89	3.38	8.90	4.60	0.214	0.026	0.001	0.807
Summer crop	10.59	9.40	12.95	10.72	0.926	0.330	0.362	0.776
Weeds	1.42	1.41	0.28	1.17	0.157	0.050	0.185	0.174
Total rotation[2]	18.48	12.78	21.85	15.31	1.002	0.162	0.013	0.832

[1] Standard Italian ryegrass; alternative: fava bean-rapeseed intercrop; [2] excluding weeds.

The 'alternative' winter crops had higher production in a single cut than the cumulated production of the two cuts of Italian ryegrass (6.75 vs. 5.63 t DM ha^{-1} respectively; *P*<0.05). The difference in forage production between years was a result of the differences in rainfall. During the 2012-13 winter season the rainfall was 1285 mm, three times the more normal rainfall of the 2011-12 winter (475 mm). There were no differences in maize production between treatments and years. The presence of weeds associated with maize showed significant differences between managements, demonstrating the herbicide effectiveness of the rapeseed crop. In both years, the AA forage had higher concentrations than SA forage of ash (100.7 vs. 78.2 g kg^{-1} DM), CP (159.6 vs. 90.6 g kg^{-1} DM), NDF (528.5 vs. 412.6 g kg^{-1} DM) and ADF (427.1 vs. 193.1 g kg^{-1} DM). The highest concentration of CP in the AA crop was due to the legume presence, while the highest proportions of NDF and ADF were the result of the high proportion of fibre of rapeseed, which induced to a lower OMD (53.2%) and energy concentration (9.1 MJ ME kg^{-1} DM) in the AA management than in SA management (80.5% and 11.9 MJ ME kg^{-1} DM respectively). No significant differences between the two types of management and years regard to maize were found. The pH value and the OM content in the soil ensure successful implementation and development of the crops in the AA management (Figure 1). However, the pH of soil in the SA management gradually declined from the beginning of the experiment. The content of available phosphorus indicates high levels of soil fertility, without significant differences between managements and years. However, the potassium level in sustainable management increased significantly during the study period. This

indicates the impact of organic fertilization as well as the extraction of K from the deeper soil layers by rapeseed.
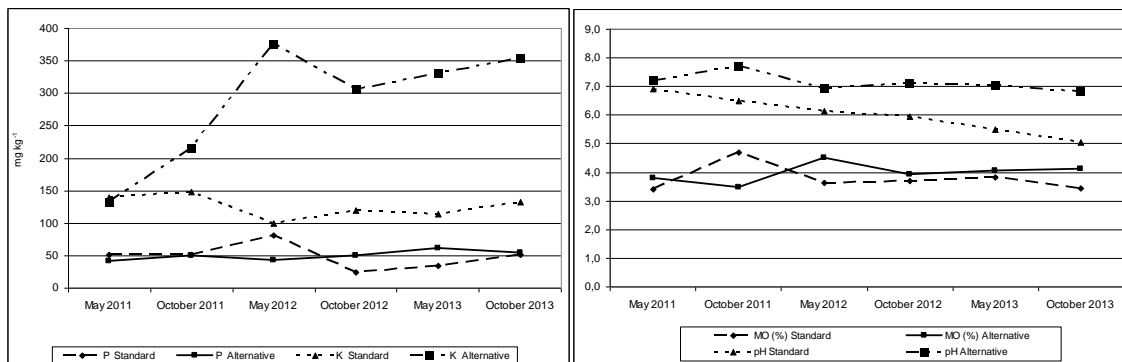

Figure 1. Changes in pH and concentrations of organic matter (OM), P and K of soil under two different managements.

Conclusions

Based on two years' results, the use of organic fertilization and the fava bean-rapeseed intercrop could be regarded as a sustainable alternative to the use of Italian ryegrass as a winter crop, and showed a substantial production of dry matter and protein. This intercropping system offers an opportunity to reduce the use of herbicides and chemical fertilizers, increase the K content and maintain a stable soil pH.

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Fava bean-rapeseed and maize silages growing under organic fertilization as a sustainable alternative for dairy cow feeding

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Abstract

The possibility of fava bean-rapeseed and maize silages grown with organic fertilization, as an alternative to Italian ryegrass and maize silages grown with conventional fertilization, were evaluated for use in the diet of grazing dairy cows. A trial was performed with 10 lactating Holstein cows divided into two groups in a 2×2 Latin Square design. Two TMR-based on Italian ryegrass and maize silages, grown using chemical fertilization (SA treatment), or fava bean-rapeseed and maize silages grown with organic fertilization (AA treatment) were compared. The results showed that there were no differences in the total dry matter intake and milk yield. However, the fatty acid profile was healthier in the AA treatment than in the SA treatment, with higher concentration of unsaturated fatty acids. The total concentrate intake was lower in the AA treatment than in the SA treatment: this treatment required 83.6 g of concentrate to produce one litre of milk, compared with 51.8 g of concentrate for the AA treatment. Using silages based on organically fertilized fava bean-rapeseed and maize in the diet of dairy cows can be an alternative to using Italian ryegrass and maize silages grown using chemical fertilization, and can reduce the required amount of concentrate in the TMR without affecting milk production.

Keywords: dairy cow, sustainability, legumes, organic fertilization

Introduction

One factor in the current crisis affecting dairy profitability is the increasing prices of agricultural commodities. This has forced dairy farmers to reduce costs and improve the efficiency of using their own forage resources. It is leading to a renewed interest in the use of cattle manure and slurry to grow forage crops, and in searching for new and more sustainable forage sources. On most dairy farms the crop rotation of maize-Italian ryegrass is repeated continuously, which demands high amounts of nitrogen fertilization and has negative effects on the soil. Manure application to crop fields to supply organic fertilization can recycle animal wastes and can be a valuable soil nutrient resource. The benefit of dairy manure application can be attributed to the improvement of physical and chemical edaphic properties (Butler and Muir, 2006). An intercropping system of fava bean (*Vicia faba* L.) and rapeseed (*Brassica napus* L.) is presented as an alternative forage system, due to the N-fixing ability of the legume and the capability of the crucifer to mobilize soil nutrients (Jiménez *et al.*, 2014). The aim of this work was to compare for grazing dairy cows, with minimal supply of concentrate in the diet, the feed intake, milk yield and milk composition of using fava bean-rapeseed and maize silages grown with organic fertilization as an alternative to using Italian ryegrass and maize silages grown with conventional fertilization.

Materials and methods

Two adjacent plots of 1.7 ha each were managed with standard (SA) or alternative approach (AA). Crops and fertilization details are described in the companion paper (Jiménez *et al.*, 2014). Ten Holstein dairy cows of 2.4±0.2 lactations (mean±SD) and with 82±11 days in milk, and 31.3±1.62 L d^{-1} of milk in the previous week to the beginning of the experiment, were selected and randomly divided into two groups. Two isoenergetic (1.41 Mcal EN$_l$ kg^{-1}DM) and

isoproteic (105.9 g CP kg^{-1}DM) total mixed rations (TMR), SA and AA, were formulated according to NRC (2001). The SA TMR included ryegrass silage (36.9% dry matter basis), maize silage (35.3%) growing both under chemical fertilization, barley straw (16.2%) and concentrate (11.7%). The AA TMR included fava bean-rapeseed silage (43.5%), maize silage (32.5%) growing both under organic fertilization, barley straw (15.6%) and concentrate (8.3%). Both TMR were offered *ad libitum* and cows were moved after evening milking until the next morning milking to a fresh paddock of 1.0 ha. Cows with production higher than 30 L per day were supplemented with an extra concentrate offered during milking sessions. After an adaptation period of 14 days, the TMR intake and milk production were recorded daily during the assay period (5 days). Grass intake was estimated by the animal performance method (Macoon *et al*., 2003). TMR was sampled daily and these samples were pooled at the end of each assay period. Milk was sampled for 3 days in both milking sessions. Grass was sampled and body weight and body condition were recorded at the first and last day in each assay period. Both TMR, extra concentrate and grass were dried at 60ºC during 24h to dry matter (DM) analysis, ground (0.75 mm) and analysed by NIRS for organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF). The fat, protein and urea content of milk samples was analysed by MilkoScan FT6000, and milk fatty acids by gas-liquid chromatography as described Chouinard *et al*. (1999). Statistical analysis was performed in SAS (1999) using the MIXED procedure for a 2×2 Latin square design.

Results and discussion

The grass had a nutritional value of 152.7 g CP kg^{-1} DM and 1.46 Mcal kg^{-1} DM. The extra concentrate was of 187.4 g CP kg^{-1} DM and 1.85 Mcal kg^{-1} DM. Table 1 shows the DM intake in both treatments.

Table 1. Dry matter intake (DMI; kg d^{-1}) under two different feeding strategies, standard (SA) or alternative (AA).

	AA	SA	s.e.	*P*
TMR intake	2.71	8.78	0.532	0.001
Extra concentrate intake	1.12	1.24	0.185	0.503
Grass intake	17.22	17.95	4.331	0.867
Total DMI	21.05	27.89	4.410	0.142

The TMR intake was lower in the AA treatment than in SA treatment (2.7 vs. 8.8 kg DM d^{-1} respectively, *P*<0.001). The intakes of extra concentrate (1.1 kg DM d^{-1}) and grass (17.6 kg DM d^{-1}) were similar in both treatments. Therefore, the nutritive value of the whole experimental diets, taking into consideration all ingredients, was similar in both treatments (144.0 g CP kg^{-1} DM and 1.47 Mcal kg^{-1} DM). Although the total DM intake in the SA treatment was higher than in the AA treatment, there were no differences between them. Consequently, no differences in live weight or body condition were observed. There were no differences in milk production (26.5 kg d^{-1}) between treatments (Table 2). According to our results, 83.6 g of total concentrate, including concentrate into the TMR and extra, was required to produce one kg of milk under the SA treatment, while the proposed alternative (AA) required 51.8 g kg^{-1} (*P*<0.001). The fat and protein contents of milk were not affected by treatments. However, the urea concentration in milk of dairy cows on the AA treatment was higher than for dairy cows feeding on SA treatment (306 vs. 214 mg kg^{-1} respectively, *P*<0.001). The unsaturated fatty acids concentration was higher in the AA treatment than the SA treatment, especially due to the higher concentration of oleic (16.88 vs. 12.96 g 100g FA^{-1}), vaccenic (1.52 vs. 0.97 g 100g FA^{-1}) and linolenic (0.59 vs. 0.34 g 100g FA^{-1}) acids (*P*<0.001) and CLA (0.59 vs. 0.37 g 100g FA^{-1}) and linoleic (0.95 vs. 0.66 g 100g FA^{-1}) acid (*P*<0.05).

Table 2. Milk production and milk composition under two different feeding strategies, standard (SA) or alternative (AA).

	AA	SA	s.e.	*P*
Milk yield (kg d^{-1})	26.09	26.96	2.297	0.709
Fat (%)	3.66	3.67	0.151	0.979
Protein (%)	3.16	3.21	0.098	0.638
Urea (mg kg^{-1})	306	214	20.0	0.001
Saturated fatty acid (g 100g FA^{-1})	76.91	82.41	0.876	0.001
Monounsaturated fatty acids (g 100g FA^{-1})	23.09	17.59	0.789	0.001
Polyunsaturated fatty acids (g 100g FA^{-1})	2.08	1.37	0.159	0.001

Conclusions

The use in dairy cow diets of fava bean-rapeseed and maize silages grown with organic fertilization can be a sustainable alternative to silage of Italian ryegrass and maize grown using chemical fertilization. Their use could reduce the supply of concentrate in the TMR, without affecting milk production. In addition, the use of slurry and manure for forage fertilization could help to reduce the costs of milk production.

Acknowledgements

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Effect of harvest and ensiling on different protein fractions in three different legumes

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Abstract

Crude protein (CP) is an important nutrient in legumes. However, during harvest and ensiling proteolysis occurs which may affect the nutritive value of legumes. Thus, the objective of the present trial was to determine the relative amount of 5 feed nitrogen (N) fractions in the first and third cut of fresh, wilted and ensiled lucerne (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*) and red clover (*Trifolium pratense*). Generally, all silages were of good fermentation quality as evidenced by the high ranking according to the Deutsche Landwirtschafts-Gesellschaft (DLG). Regardless of the cuts, during wilting and ensiling the relative amount of the non-protein N (A), slowly degradable (B_3) and undegradable fractions (C) increased, whereas that of the fast (B_1) and variably degradable (B_2) fractions decreased. Compared with red clover and sainfoin, the proportion of fraction A was greater and that of fractions B_2 and B_3 were lower in lucerne. These differences among legume species might be an effect of plant secondary compounds present in red clover and sainfoin.

Keywords: legumes, silage, wilting, protein fractions

Introduction

During harvest and ensiling the crude protein (CP) fraction undergoes a degradation thereby altering the CP availability and overall nutritive value of legume forages. As a result of proteolysis and desmolysis, undesired substances such as biogenic amines are formed, which ultimately might impair animal health status. Licitra *et al.* (1996) proposed a fractionation method for CP. The non-protein nitrogen (NPN) was denoted as the A fraction, while the true protein was divided into B_1 (buffer soluble CP = fast degradable), B_2 (buffer insoluble CP = variable degradable), B_3 (CP insoluble in neutral detergent = variable to slow degradable) and C (CP insoluble in acid detergent = indigestible) fractions based on decreasing solubility. As reviewed by Hoedtke *et al.* (2012) extrinsic and intrinsic factors influence the relative proportion of the different protein fractions. The aim of this study was therefore to monitor changes in the relative abundance of the five protein fractions in lucerne (LU), red clover (RC) and sainfoin (SF) just after cutting in the fresh state, after 1 d of wilting and after ensiling. This was repeated twice, in early summer and autumn (cuts 1 and 3, respectively).

Materials and methods

The LU, RC and SF were cultivated as pure swards in Posieux (altitude 650 m a.s.l.). In July and September 2012, fresh and wilted samples were collected at three different locations on the field on the day of cutting and 1 d after cutting, respectively. In addition, from the same location wilted legume samples were collected and ensiled for 86 and 95 d in 1.5 L laboratory silos. In the forage samples, the relative amount of the 5 fractions were analysed according to Licitra *et al.* (1996). In addition, in the silages, dry matter (DM), pH, NH_3 content and fermentation products were determined. Data were analysed using analysis of variance with legume species (LU, RC, SF), cutting number (cut 1 and 3) and time of sample collection (fresh, wilted and ensiled) and the 2- and 3-way interactions as fixed factors (SYSTAT 13).

Results and discussion

After mowing, the DM content of the legumes ranged from 14 to 18%. After 1 d of wilting, the DM content of the first cut was greatest in LU (48%) followed by SF (39%) and RC (34%). For

the third cut, the LU and SF DM content was similar (32% for both) but lower compared to RC (36%). The DM content of the silages remained comparable in order and magnitude as the wilted LU, SF and RC and were 49, 37 and 33% in the first cut and 31, 31, and 34% in the third cut, respectively. Based on the DLG evaluation scheme (Staudacher and Schenkel, 2007) one LU batch of the first cut was evaluated with only 73 points whereas all other legume silages ranked between 96 and 100 points, implying a very good fermentation quality. The main reason for the lower score of the LU batch was a higher acetic acid content, but the quality could still be regarded as good. Expressed per total N, the proportion of ammonia N in the LU, RC and SF amounted to 8.4, 7.3 and 5.5% in the first cut and 10.9, 7.0 and 4.6% in third cut, respectively ($P < 0.01$). Regardless of cutting number, CP content of the fresh LU were similar ($P = 1.00$). By contrast, CP content of the RC and especially of the SF were greater in the third than the first cut ($P < 0.01$) (Figure 1a). Due to the fermentation activity and degradation of sugars, CP content was generally greater in the silages as in the fresh grass (Figure 1a).

In Figure 1 b-f, the relative amounts of fraction A, B_1, B_2, B_3 and C expressed as percentage of total CP of fresh, wilted and ensiled LU, RC and SF harvested in early summer (first cut) or autumn (third cut) are presented. The relative amount of NPN increased during the wilting and especially during the ensiling process (Figure 1b). The greatest changes were observed in LU where the relative amount of fraction A in the silages was 60% greater compared to RC and SF ($P < 0.01$). A possible explanation that this shift was most evident in LU might be the fact that condensed tannins in the SF or products of the polyphenol oxidase of the RC could have hindered proteolysis of CP, resulting in lower amount of fraction A. Similarly, Tabacco *et al.* (2006) showed that the addition of chestnut-tannin to lucerne reduced proteolysis and consequently the amount of NPN in silages.

Regardless of cut number, the relative amount of fraction B_1 and B_2 decreased during the wilting and ensiling process ($P < 0.01$) (Figure 1c and d). The relative proportion of fraction B_3 was greater in wilted than fresh LU and RC, but again lower in the silages ($P < 0.01$). In the third cut, the same was observed also for SF whereas in the first cut the relative amount increased from fresh to the ensiled samples (Figure 1e). In LU and RC the relative amount of fraction C was low in both cuts and only minimal changes were observed between fresh, wilted and ensiled samples (Figure 1f). In the first cut the relative amount of fraction C was greater than in the third cut and was greater in wilted than fresh SF with intermediate values for ensiled SF (Figure 1f).
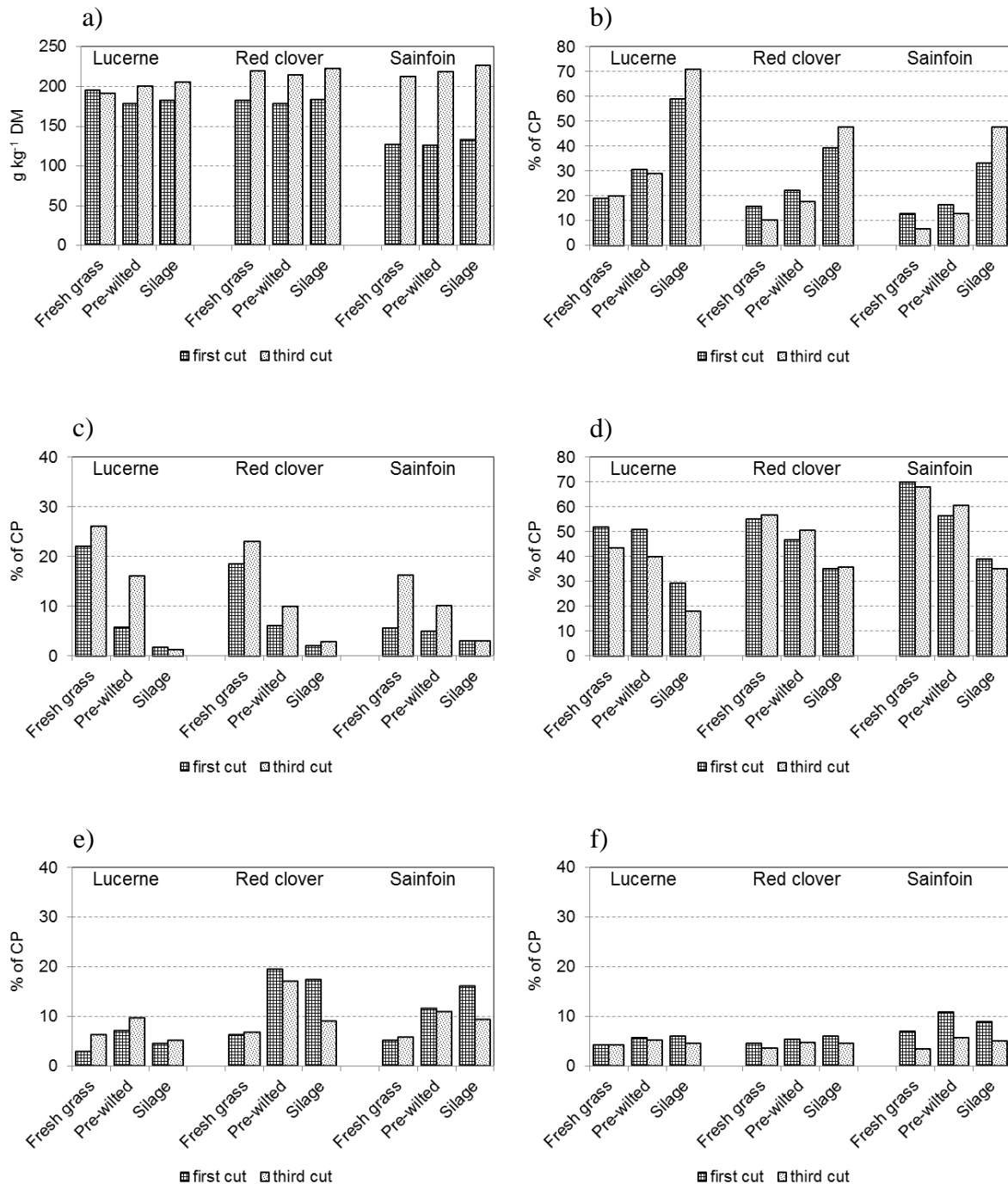
Figure 1: Crude protein content (a) and relative amount (expressed in percentage of total crude protein) of fraction A (b), B_1 (c), B_2 (d), B_3 (e) and C (f) in fresh, wilted and silages of lucerne, red clover and sainfoin harvested in early summer (first cut) and autumn (third cut). Values are least square means of 3 batches collected in the field at different locations.

Conclusions

The relative proportion of the CP fraction changed during the process of harvesting and conservation resulting in a marked shift towards a greater amount of NPN and a marked decrease of the fast and variable degradable true protein fraction.

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Nutritive value evaluation of some grasses and legumes for ruminants

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Abstract

The experiment was conducted at the experimental field of the Institute for Animal Husbandry in the vicinity of Belgrade, in a randomized block design with four replications. The study included four grass species: tall fescue (*Festuca arundinaceae* Schreb.), perennial ryegrass (*Lolium perenne* L.), cocksfoot (*Dactylis glomerata* L.) and meadow fescue (*Festuca pratensis* L.) and two legumes: red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.). The aim of this study was to compare the nutritional value of grasses and legumes that are commonly used in the Republic of Serbia for feeding of livestock. Lucerne had the highest content of CP (158.87 g kg^{-1}), and ryegrass the highest ME content (9.83 MJ kg^{-1} DM). NEL content of the studied species was insufficient to meet the lactating requirements of cows, so that diets for cows should consist of forage and concentrated feed.

Keywords: grass, legume, nutritive value

Introduction

Contemporary livestock production, especially the production of meat and milk from ruminants, is based on a combined diet containing roughage and concentrate. Lucerne hay is the most commonly used fodder in lowland regions due to its high productivity, resistance to high and low temperatures, uniform yield during the growing season and longevity. In the hilly and mountainous areas, in addition to lucerne hay, red clover hay and, in a small proportion, hay of grass-legume mixtures are used. Grass-only crops are very little used in the diet. In contrast to Serbia, in north-western Europe ryegrass has a more significant role in livestock production due to its high production and high nutritional value (Losche *et al*., 2008). Also, Lättemäe and Tamm (1997) report that grasses are the most important and basic fodder for dairy cows in Estonia. In their studies, it was suggested that grass-legume mixtures have higher contents of metabolic energy (of 10.6-11.00 MJ kg^{-1} DM) compared to that of grasses (10.3-10.6 MJ kg^{-1} DM), but the differences were not statistically significant. Thompson (2013) has concluded by comparing the nutritive value of tall fescue and cocksfoot in monoculture and in mixture, that the grasses in monoculture have similar nutritional value, but when mixed with lucerne, tall fescue shows better results. The aim of this study was to compare the nutritional value of certain grasses and legumes that are commonly used in Serbia in the diet of cattle, both in monoculture and in mixture.

Materials and methods

The trial was conducted at the experimental field of the Institute for Animal Husbandry in the vicinity of Belgrade (44° 49' 10" N 20° 18' 45" E) in a randomized block design with four replications. The main plot size was 5 m^2. The study included four grasses: tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), cocksfoot (*Dactylis glomerata* L.) and meadow fescue (*Festuca pratensis* L.) and two legumes: red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.). Sowing was carried out in 2009, and the study was done in 2010. The soil on which the trial was established is the low carbonate chernozem, of favourable water and air regime.

For this study samples of plant material from the first cut in four replications were used. The samples were dried at a temperature of 60 °C for 72 hours, and then milled and sifted through a 1 mm sieve. The crude protein content was determined using the Kjeldahl method, the content of crude fat according to AOAC method (1990), ash content by burning at 525 °C, and the content of NFE was calculated. For calculation of metabolic energy, and the net energy for lactation the following formulas were used (Nauman and Bassler, 1993; Baranauskas *et al.*, 1998):

ME=14.07+(0.0206xCf)+(0.0147xCF)-(0.0114xCP)±4.5%;

NEL=9.10+(0.0098xCf)-(0.0109xCF)-(0.0073xCP),

Cf- crude fat g kg^{-1}; CF- crude fibre g kg^{-1};CP- crude protein g kg^{-1}

The data were analysed by one way ANOVA (analysis of variance). The significance of the mean values was performed using the LSD test (StatSoft, Inc., 2007).

Results and discussion

Results of chemical analysis and the content of metabolizable energy (ME) and net energy for lactation (NEL) are given in Table 1. The content of crude protein (CP) was significantly higher in legumes compared to the grass species. Lucerne had the highest content of CP, 158.87 g kg^{-1}. Among the examined grasses the highest content of CP was in cocksfoot, 126.25 g kg^{-1}. Aufrere *et al.* (2008) also concluded that cocksfoot has the highest content of crude protein of all tested grasses. CF content also showed significant variation ($P<0.01$) among the examined species. Cocksfoot had the highest CF content. No statistically significant differences in the content of CF were established between the species tested. Other studied parameters showed significant ($P<0.05$) variation depending on the species. Ryegrass had the highest ME content of 9.83 MJ kg^{-1} DM, which was not significantly different from the ME content of red clover and meadow fescue. The lowest content of ME was recorded for lucerne (8.94 MJ kg^{-1} DM).

Table 1. Chemical characteristics (g kg^{-1}DM), level of metabolic energy and net energy for lactation (MJ kg^{-1} DM) of grass and legumes. (CP-crude protein; CF-crude fibre; Cf- crude fat; Nfe-nitrogen free extractives; ME-metabolic energy; NEL-net energy for lactation).

Species	CP	CF	Cf	Ash	Nfe	ME	NEL
Tall fescue	93.65[d]	284.42[ab]	22.85	53.05[c]	469.12[a]	9.29[b]	5.54[b]
Perennial ryegrass	116.90[cd]	237.40[d]	28.22	73.52[b]	450.70[a]	9.83[a]	5.94[a]
Cocksfoot	126.25[bc]	288.12[a]	27.50	55.15[c]	428.12[a]	8.96[b]	5.30[b]
Meadow fescue	104.17[cd]	278.02[ab]	24.70	70.10[b]	443.45[a]	9.30[ab]	5.55[b]
Red clover	147.90[ab]	241.00[cd]	28.15	77.10[b]	427.37[a]	9.42[ab]	5.67[ab]
Lucerne	158.87[a]	262.32[bc]	26.20	89.58[a]	375.57[b]	8.94[b]	5.33[b]
Level of significance	**	**	ns	**	*	*	*

Fulkerson *et al.* (2007), in their study, also show results that lucerne is characterized by the lowest content of ME when compared to other tested grasses and red clover. Content of NEL was also significantly higher in perennial ryegrass (5.94 MJ kg^{-1} DM) in comparison with other grasses and lucerne. Our research confirms the results of Kohoutek *et al.* (2007) who studied the quality of individual varieties of perennial ryegrass, cocksfoot and meadow fescue, and show that the content of NEL in varieties of perennial ryegrass are slightly higher 5.73-5.85 MJ kg^{-1} DM, compared to varieties of cocksfoot (5.72-5.77) and meadow fescue (5.70-5.75). NEL requirements of dairy cows during the lactation period are different. In early lactation the NEL requirement amounts to 6.9-7.4 MJ kg^{-1} DM, in the middle of lactation 6.6-6.9 MJ kg^{-1} DM, and at the end of lactation 6.4-6.6 MJkg^{-1} DM (Jovanovic *et al.,* 2000). Content of NEL, as the primary limiting factor in the production of milk, in the studied species of grasses and legumes, is inadequate to meet the needs of lactating cows. This deficiency in regard to NEL requirement of 6.4 to 7.4 MJ kg^{-1} DM should be compensated through supplementation with concentrated feed, especially at the beginning of the lactation period.

Conclusion

Based on the study of the quality of four grasses and two legumes for ruminant nutrition, as expected, a higher content of crude protein (CP) was obtained in legumes compared to grass species. Lucerne had the highest CP content of 158.87 g kg^{-1}, while the perennial ryegrass had the highest ME content of 9.83 MJ kg^{-1} DM. NEL content was also significantly higher in the ryegrass (5.94 MJ kg^{-1} DM) compared to other grasses and lucerne. NEL content of the studied species was inadequate to meet the requirements of lactating cows, so the diets for cows should consist of forage and concentrated feed. Monocultures of the species tested, in regard to the quality, do not meet the needs of ruminants for nutrients and energy, so the combined cultivation of grass-legume mixtures is more useful.

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Forage quality in legumes and non-leguminous forbs

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Abstract

As data for non-leguminous broad-leaf grassland species are scarce, the aim of this study was to obtain novel information on forage quality components and yield for a number of non-leguminous forb and legume species compared to a grass-clover mixture. Four non-leguminous forb species, three legumes and a perennial ryegrass-white clover mixture were investigated in a cutting trial (randomized block) with four harvests (May-Oct) during 2009 and 2010. In pure stands, legumes outyielded non-leguminous forbs for protein content. Ribwort plantain and lucerne had higher ADF and lignin concentrations, but in other species ADF and lignin contents were equal to that of grass-clover. More research is needed to explore the potential value of non-traditional species in the forage-feed food chain.

Keywords: herbs, forbs, legume, herbage quality

Introduction

Fresh herbage is an important natural source of nutrients in diets of ruminants and horses. Most studies on forages have been carried out with agronomical important grass species, such as perennial ryegrass, and with legume species such as white clover, but few studies investigated other grassland forage species. As data of dicotyledonous species grown in a sward are scarce, yield and quality in a number of non-leguminous forbs and legume species were compared with a grass-clover mixture to get an insight into species differences and seasonal variation.

Materials and methods

Pure stands with each of four non-leguminous forb species, i.e.: salad burnet (*Sanguisorba minor*), caraway (*Carum carvi*), chicory (*Cichorium intybus*) and ribwort plantain (*Plantago lanceolata*), and three legume species, i.e.: yellow sweet clover (*Melilotus officinalis*), lucerne (*Medicago sativa*) and birdsfoot trefoil (*Lotus corniculatus*) were sown, plus a commercial mixture (85% *Lolium perenne* + 15% *Trifolium repens*). In addition, chervil (*Anthriscus cerefolium*) was sown but only produced herbage in the first cut, and unreplicated plots were sown with borage (*Borago officinalis*) and viper's bugloss (*Echium vulgare*); these species were excluded from statistical analyses. Net plot size was 1.5 m × 9 m. Swards were cut with a forage harvester on 29 May, 9 July, 21 August and 23 October 2009 and on 31 May, 13 July, 19 August and 21 October 2010. After cutting, samples of the harvested herbage were taken to determine DM content and quality parameters (ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL)) (Elgersma *et al*., 2014). The experimental design was a randomized complete block with two replications. There were eight 'species' (the seven forb species plus the mixture) and four harvests per year. Analysis of variance procedures were applied using the MIXED procedures of SAS. Yield and quality compounds were evaluated with a model that included fixed main effects of species, harvest date and their interaction. All tests of significance were made at the 0.05 level of probability.

Results and discussion

Average yields ranged from 3.9 to 15.4 t DM ha^{-1} year^{-1} and were lower in yellow sweet clover, salad burnet and caraway than in lucerne and the grass-clover mixture. Yields were lowest in the fourth harvest ($P < 0.001$). The seasonal growth pattern was very different in both years: in

2009, the first cut yielded most, whereas in 2010 the second cut was most productive, except in caraway. Annual yield was lower in 2010 than in 2009 for most species except caraway. Due to this variability there was no overall effect of year. For most species the DM yield of the first cut was much higher in 2009 than in 2010: e.g., that of grass-white clover yield was 5.3 and 2.4 t DM ha^{-1} and birdsfoot trefoil was 4.8 and 2.6 t DM ha^{-1}, respectively (Elgersma *et al*., 2014). In pastures, species are mixed but here pure stands of forb species were studied. Caution should be taken when intrapolating yield data.

The weather may provide an explanation for the fact that yields in the first harvest were much lower in 2010 than in 2009. First of all, the winter of 2009/2010 was severe and spring growth started late. The average temperature in April 2010 was only 6.5 °C whereas in April 2009 it was 9.4 °C. Also, in May 2010 the air temperatures were lower than in May 2009. Therefore, the effective primary growth period differed. This implies that the forage was probably less mature on 31 May 2010 than on 29 May 2009.

Very high concentrations of ash (> 150 g kg^{-1} DM) were found in borage and viper's bugloss, both of which have cuticular hairs (Figure 1a). These species had a very low hemicellulose (i.e., NDF – ADF) content, whereas that of chervil was very high (114 g kg^{-1} DM). Chervil also had a numerically high fatty acid concentration in the first cut (30 g kg^{-1} DM) compared to birdsfoot trefoil (28 g kg^{-1} DM in the first cut), which had a higher content ($P < 0.01$) than other species (Elgersma *et al*., 2013; Figure 1b).

Among the replicated species, all parameters showed significant differences ($P < 0.001$) (Elgersma *et al*., 2014). Averaged across harvests and years, ribwort plantain and lucerne had the highest concentrations of NDF, ADF and ADL (Figure 1b). Birdsfoot trefoil had low NDF and ADF concentrations but a high ADL concentration and thus a high lignification of the cell wall, as well as a low ash content. The highest ash concentration (143 g kg^{-1} DM) was found in chicory. The crude protein concentration was highest in the three legume species and in the grass-clover mixture, and lowest in chicory and plantain. The concentration of 'other compounds' including water-soluble carbohydrates was significantly higher in salad burnet than in all other species; it was also higher in chicory and caraway than in the legume species and the mixture (Figure 1b).

Among the replicated species, there were differences between harvests ($P < 0.001$) for all parameters. Species × cut interactions were not significant for yield and most quality compounds, except for concentrations of crude protein and ash ($P < 0.05$) (not shown). There were no effects of year, except for lignin content ($P < 0.05$).

Large differences were found for ash and CP contents between harvests ($P < 0.001$). Ash concentrations were lower in cuts 1 and 2 than in cuts 3 and 4, and increased from the second cut onwards, whereas CP concentrations were higher in the first than in the second cut and then increased to become highest in cuts 3 and 4. There were large differences ($P < 0.001$) between harvests in the concentration of 'other compounds' including water-soluble carbohydrates, which were highest in the first cut and lowest in the third cut. The fibre contents (i.e., concentrations of NDF, ADF and ADL) differed between harvests ($P < 0.001$) and were lowest in the fourth harvest for all species. The concentrations of ADF and its components (hemicelluluse and lignin) were higher in 2010 than in 2009 ($P < 0.05$). No other effects of year on forage quality compounds were found. parameters. Species × cut interactions were not significant for yield and most quality compounds, except for concentrations of crude protein and ash ($P < 0.05$) (not shown). There were no effects of year, except for lignin content ($P < 0.05$).

Figure 1. Concentrations of quality compounds in (left) chervil (Ch) in the first cut, borage (Bo) in cuts 2 and 3 in
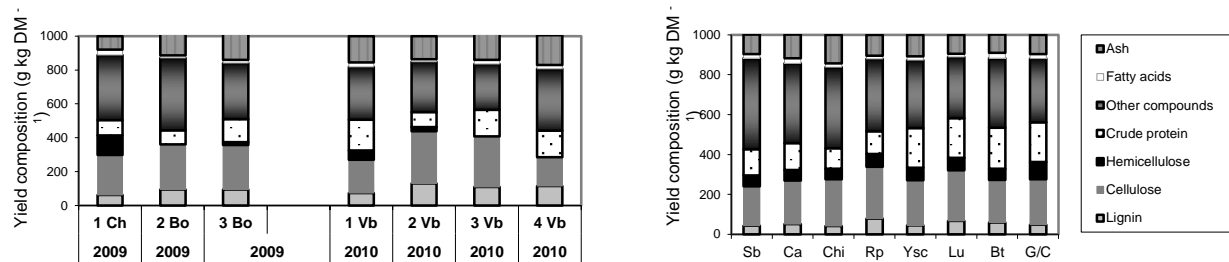
2009 and vipers' bugloss (Vb) in cuts 1 to 4 in 2010, and (right) salad burnet (Sb), caraway (Ca), chicory (Ch), ribwort plantain (Rp), yellow sweet clover (Ysc), lucerne (Lu), birdsfoot trefoil (Bt) and grass-clover (G/C), averaged across 4 harvests and 2 years. (Modified from Elgersma *et al*., 2014.)

Large differences were found for ash and CP contents between harvests ($P < 0.001$). Ash concentrations were lower in cuts 1 and 2 than in cuts 3 and 4, and increased from the second cut onwards, whereas CP concentrations were higher in the first than in the second cut and then increased to become highest in cuts 3 and 4. There were large differences ($P < 0.001$) between harvests in the concentration of 'other compounds' including water-soluble carbohydrates, which were highest in the first cut and lowest in the third cut. The fibre contents (i.e., concentrations of NDF, ADF and ADL) differed between harvests ($P < 0.001$) and were lowest in the fourth harvest for all species. The concentrations of ADF and its components (hemicelluse and lignin) were higher in 2010 than in 2009 ($P < 0.05$). No other effects of year on forage quality compounds were found.

Lucerne, chicory, ribwort plantain and birdsfoot trefoil, in single-species stands, had similar DM yield to a perennial ryegrass-white clover mixture. The nutritional benefits of various forbs may encourage adoption of these species by farmers, but from a management viewpoint they must be balanced against the lack of persistence of most forbs in mixed swards under cutting / grazing.

Conclusion

Various forbs had a relatively high nutritive value (the legumes had a high protein content, salad burnet had a low NDF content and a high proportion of other compounds including water-soluble carbohydrates) and could enhance the nutritional profile of mixed-species pasture swards.

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Feed value of restrictedly and extensively fermented organic grass-clover silages from spring and summer growth

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Abstract

The spring and the summer growth of an organic grass-clover sward were preserved as extensively and restrictedly fermented silages in laboratory silos.The aim was to develop and test the hypothesis that such crops contribute complementary energy and protein qualities that can be exploited in mixed rations. The summer growth, containing 76% red clover, contributed more, and more stable crude protein than the spring growth, which was dominated by grasses. Nevertheless, when preserved as silage, summer growth supplied less metabolizable protein and net energy lactation because of its lower digestibility. Lower feed value remains to be validated in feeding experiments, and the quality of regrowth silages may also be improved by more frequent or appropriate timing of harvests. Restricted fermentation obtained by application of formic acid improved energy and protein preservation.

Keywords: digestibility, metabolizable protein, net energy lactation, red clover, soluble protein

Introduction

In grass-clover swards grown at high latitudes and with low external N supply, there are usually considerable differences in the content of legumes between spring and summer growths. Forage from the first cut is dominated by grass species and has a lower content of crude protein (CP) and an easily digestible carbohydrate fraction (Steinshamn and Thuen, 2008). In contrast, the yields from regrowths contain more legumes, more indigestible cell walls and more CP. At feeding, these qualitatively different forages might be utilized complementarily, according to the animal demands. To realize their potential alone or together and explore their complementarity, their respective values as protein and energy sources have to be determined. Knowing that energy preservation and prevention of protein degradation during wilting and ensiling are critical for the final feed value, we investigated gains and losses obtained by restricted versus extensive fermentation of the crops. The initial results presented here express feed value according to NorFor (Volden, 2011).

Material and methods

The plant material was harvested from a mixed crop of *Phleum pratense, Festuca pratensis, Lolium perenne, L. boucheanum* and *Trifolium pratense*. The spring growth was harvested at late stem-elongation of the dominant grass species, *P. pratense*, and the summer growth 614 d° afterwards (base temperature 0 °C). The content of *T. pratense* was 30% of DM in spring and 76% in summer growth.

The herbage was wilted indoors for 24 hours before ensiling with different types of additives (4 ml kg FM[-1]) in evacuated and sealed polyethylene bags. The additives were: 1) water (Control treatment (C)), 2) formic acid (FA, 850 g kg^{-1}), and 3) lactic acid bacteria (LAB) (Kofasil® Lac, Addcon Europe). The dosage of LAB corresponded to 10^5 cfu per g FM.

Dried (60 °C) samples of herbage and silages were analysed for ash, CP, buffer soluble CP (sCP) (Hedquist and Uden, 2006), neutral detergent fibre (NDF) (Mertens *et al*., 2002) and

water-soluble carbohydrates (WSC) (Larsson and Bengtsson, 1983). Freshly frozen silage samples were analysed for pH, and content of organic acids and ethanol (Ericsson and André, 2010) and NH_4-N. The oven DM contents of the silages were corrected for volatile losses according to NorFor. Concentrations of indigestible NDF (iNDF) were determined by a 288 h *in situ* incubation (Huhtanen *et al*., 1994). Organic matter digestibility (OMD) was calculated from iNDF and NDF concentrations (Huhtanen *et al*., 2013). Net energy lactation (NEL_{20}), metabolizable protein (MP, calculated as amino acids absorbed in the small intestine (AAT_{20})) and protein balance in the rumen (PBV) were calculated according to NorFor at daily intake of 20 kg DM (Volden, 2011). The constituents in herbage were modelled using the procedure MIXED in SAS (SAS Institute Inc., 1999) with growth (spring or summer) and wilting as fixed factors and replicate (1-3) as random. For silages, the model included growth and silage additive as fixed factors and replicate (1-3) as random.

Results and discussion

The crop harvested from the spring growth was more digestible and contained less CP and more WSC than the summer growth, which was dominated by mature red clover (Table 1). All silages were well fermented as evaluated from their pH and the concentration of NH_3-N. According to the content of organic acids, addition of LAB caused the most extensive fermentation, and FA the least. Protein solubility increased during wilting in the case of the spring growth, but not in summer growth (Table 1).

Table 1. Organic matter digestibility (OMD) and content of dry matter (DM), ash, crude protein (CP), soluble CP (sCP), water soluble carbohydrates (WSC), neutral detergent fibre (NDF) and indigestible NDF (INDF) in a fresh and wilted grass-clover crop harvested in spring and summer growth. N=3.

Herbage, growth (G) and wilt (W)	DM g kg^{-1}	Ash	CP	sCP	WSC	NDF	iNDF	OMD
		g kg DM^{-1}						
Spring growth, fresh	163	70	101	32	264	391	59	769
Spring growth, wilted	235	76	114	39	221	396	67	759
Summer growth, fresh	139	99	133	40	112	361	113	707
Summer growth, wilted	232	102	138	40	87	383	121	695
SEM	5.0	1.5	2.8	0.9	5.3	7.3	3.0	4.0
Sign. effect of ($P \leq 0.05$)	G, W	G, W	G, W	G, W, G×W	G, W	G	G, W	G, W

The CP fraction was further solubilized during fermentation (Table 2), and the soluble proportion was higher in silages from spring growth than in silages from summer growth ($P<0.001$), and higher in extensively fermented (C, LAB) compared to restrictedly fermented (FA) silages ($P<0.001$). The restriction of silage fermentation caused by application of formic acid contributed positively to energy preservation and protein feed value (NEL_{20}, MP), compared to treatments causing more extensive fermentation (Table 2).

Table 2. Organic matter digestibility (OMD) and content of dry matter (DM), ash, crude protein (CP), soluble CP (sCP),) metabolizable protein (MP), protein balance in the rumen (PBV) and net energy for lactation (NEL_{20}) in wilted silages made from spring and summer growth of a grass-clover crop and according to type of additive.

Silage, growth and additive	DM g kg^{-1}	Ash	CP	sCP	OMD	MP	PBV	NEL_{20} MJ kg DM^{-1}
		g kg DM^{-1}						
Spring growth (n=9)	246	84	124	72	760	80	7	5.9
Summer growth (n=9	237	111	155	70	699	67	56	5.2
SEM	4.9	1.0	3.5	1.9	3.0	0.4	3.4	0.03
Significance, $P\leq$	NS	0.01	0.01	NS	0.001	0.001	0.01	0.001
Control (n=6)	243	99	142	77	731	69	41	5.5
Formic acid (n=6)	240	97	137	62	729	83	13	5.7
Lactic acid bacteria (n=6)	242	98	140	76	729	69	41	5.6
SEM	4.5	0.9	3.4	2.0	3.1	0.4	3.1	0.04
Significance, $P\leq$	NS	0.05	NS	0.001	NS	0.001	0.001	0.05

Conclusions

Fresh and wilted herbage from a summer growth, dominated by red clover, contributed more, and more stable CP than that of the spring growth, which was dominated by grasses. Preserved as silage, it still supplied less metabolizable protein because of its lower digestibility. Whether it has a potential as a complementary forage to silages from spring growth with a lower CP content needs to be evaluated *in vivo*. Further investigations will reveal if the quality of summer growth silages may be improved by more frequent or appropriate timing of harvests.

Acknowledgements

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Feeding, mycological, and toxicological quality of haylage

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Abstract

The objective of this study was to detect *Fusarium* spp. and mycotoxins contents in plant material and haylage from permanent grasslands. Feeding quality was also evaluated. Quality and mycotoxin content were different in various level of haylage bales. Parameters of some upper and middle layers did not correspond to proper lactic acid preservation, and these samples were classified as harmful to health and not usable for feeding.

Keywords: permanent grassland, haylage, *Fusarium*, quality, mycotoxins

Introduction

One of the factors which can negatively influence the quality of roughage relates to microorganisms which can colonize these materials. Mycobiota in haylage produced from various types of grass stands were examined during research focused on the presence of potential pathogenic and allergenic microorganisms, and in particular *Fusarium* spp. Two locations were chosen, with various types of grass stands used for producing haylage. Location 1 (ca 0.23 ha in total) lies in the foothills of the Hostýn Hills of eastern Moravia in the Zlín District (330–365 m a.s.l.). The grass stand at this location previously had been used to produce hay, and it currently serves for haylage production. Location 2 (ca 0.57 ha) lies in the southeast of the Czech Republic within the Nový Jičín District (281 m a.s.l.). The stand at this location was established in 2010 in an environmentally friendly farming system as a clover–grass mixture on arable land. In future, it will be used over the long term as permanent grassland. In the past, this plot was used as arable land. No intensification practices are applied to the stands at either location, and the haylage production is used within the same farming operations for feeding beef cattle (Charolais, Piedmontese and Limousin breeds).

From a phytocoenological viewpoint, the chosen locations are markedly different. Location 1 has the character of a permanent grassland of the mountain meadow type, with the predominant species being members of the Poaceae (52%) and high representation of herbs (29%) and some clovers (12%). By contrast, the stand at Location 2 is characterized by a high proportion of red clover (75%) and annual ryegrass (23%); there are only scattered covering of other Poaceae species and the proportion of herbs is minimal, mainly weed species (*Taraxacum* sect. *Ruderalia*, *Rumex* spp., *etc.*) that are gradually replacing the dominant species. The objective of this study was to evaluate the feeding parameters and mycological and mycotoxicological quality of roughage production in an experiment involving simulated surface damage to wrapped hay bales.

Materials and methods

Green mass collection was undertaken at each location prior to the first and second cuttings. From each location, four representative partial samples were collected (random collection diagonally across the plot). Airborne microflora collection was undertaken while collecting the green mass. In the location of the green mass collection, in a square area of 0.5 m^2, were placed diagonally opposite one another two Petri dishes with surface areas of 10 cm^2 and containing a potato dextrose agar medium. Exposure time was 10 minutes.

The technology used for the production of the haylage bales was different at the two monitored locations. The haylage was produced from the first mowing. The project's designers

intentionally left the production of haylage fully in the hands of the specific agricultural company which was farming each monitored location, the objective being to have the quality of haylage subsequently used for the research as similar as possible to that produced in actual farming practice. At Location 1, the material was pressed and wrapped in bales with a high dry matter content in the cured fodder, which subjectively can be said to have resembled hay. By contrast, at Location 2, after mowing, the green mass was left to cure for only a short time (spreading it out and once turning it over) and then baled at a low dry matter content either that same evening or the following morning.

From each monitored location, two bales of haylage were acquired for further analysis. The first was left in an undisturbed state as a control. The second bale had the plastic covering artificially broken in several places with the tines of a pitchfork (simulating damage from branches) or sliced with a knife (simulating greater mechanical damage). To simulate water leaking into the bale, at 10-day intervals water (10 L per bale) was poured over the bales.

Individual samples of the haylage were collected at one time on a date approximately 90 d after the bales were wrapped. From each bale, 3 mixed samples were collected from three different layers: the edge of the bale, the middle layer of the bale, and the centre of the bale. From each sample, three partial samples were collected at random at the edge of the given layer. The minimum weight of one mixed sample was 2 kg. The presence of *Fusarium* spp. was morphologically evaluated in detail, and within the samples there were morphologically determined also other genera of microscopic fungi. The isolated *Fusarium* spp. were preserved in a cultures collection for subsequent species identification. Deoxynivalenol, T-2 toxin, zearalenone, fumonisin, and aflatoxin content were analysed using enzyme-linked immunosorbent assay (ELISA). Comprehensive feed analysis and sensory evaluation of the produced fodder were undertaken.

Results and discussion

Green mass. A greater number of *Fusarium* CFU was determined in the second collection at both locations. The difference between the collections in May and August was statistically significant ($P = 0.04$) and no effect of the location on the occurrence of detected fungi was found ($P = 0.18$). Even though fewer *Fusarium* spp. were present at Location 2, the reason clearly lies in the composition of the grasslands. While in this location grass species constitute only 23% of the stand, at Location 1 they are the dominant component (60%) of the stand. It is known from the scientific literature that fungi of the genus *Fusarium* occur abundantly in Poaceae plants and in some cases they may remain active even in wintering grass stands.

Haylage samples. *Fusarium* spp. were found only in bales which had been artificially damaged, and only from the uppermost layers. The greatest numbers of CFU of this genus were found in samples taken from Location 2 (2391 CFU g^{-1} DM). At Location 1, the quantity of CFU was lower (111 CFU g^{-1} DM). *Fusarium* spp. were not found in the samples of high-quality haylage (Figure 1). The large difference between locations in the high-quality haylage can be explained in part by the extent of damage to the bales.
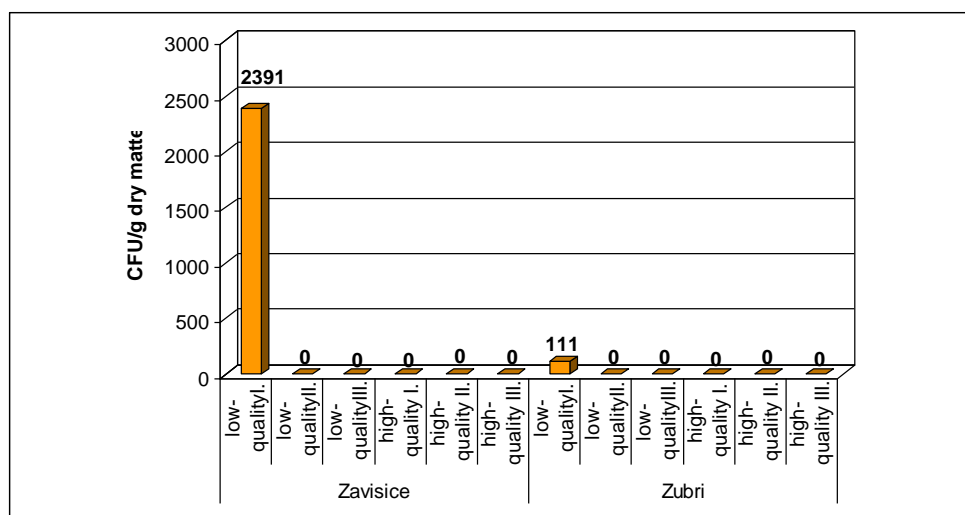
Figure 1. Occurrence of *Fusarium* fungi (CFU / g dry matter) in haylage samples

Qualitative parameters and concurrent sensory evaluation are summarized in Table 1. There was evidence of very low quality in several samples of haylage, mostly owing to the mechanical damage to the bales.

Table 1. Feed analysis – selected parameters

sample	NL (g/kg DM)	SNL (g/kg DM)	Fibre (g/kg DM)	ADF/NDF (%)	Lactic acid (g/kg FM)	Acetic acid (g/kg FM)	Butter acid (g/kg FM)	pH
1	128.8	81.14	326.27	63.09/38.13	17.3	1.8	1.5	5.5
2	159.65	100.58	310.74	59.40/35.83	20.4	2.9	1.0	5.1
3	146.79	92.48	299.25	55.41/36.05	19.6	3.9	1.3	5.2
4	239.1	155.42	210.72	45.8/48.65	1.5	0.3	0.0	8.4
5	137.18	86.42	382.27	60.74/47.89	5.3	5.8	0.6	8.7
6	139.61	87.96	326.39	61.11/41.20	20.6	5.9	1.0	5.6
7	97.98	48.99	290.83	59.62/36.95	12.9	4.1	1.6	5.5
8	116.93	80.68	297.68	61.15/37.81	18.7	4.7	0.7	4.9
9	99.95	49.97	292.89	62.23/41.49	13.6	4.7	0.5	5.2
10	289.39	188.1	118.86	55.32/53.09	1.0	1.0	0.0	8.4
11	157.56	99.26	347.31	67.39/55.73	1.3	5.9	0.0	9.2
12	106	53	271.63	53.05/41.69	9.2	7.3	0.0	4.9

The haylage from Location 2 (samples 1−3) was all evaluated as suitable for feeding, although the overall level of the fodder was rated as less than satisfactory. It is clear that as the fermentation process had progressed, the pH levels and acid content had developed accordingly. Nitrogen content, fibre, and other content parameters depended on the species composition of the grass stand. An identical source of green matter produced entirely different results when stored in a mechanically damaged bale (samples 4−6). In the upper layer of the bale (sample 4), the fermentation process and lactic acid preservation did not occur; the fodder was evaluated as unsatisfactory and even harmful to health and with a high content of macroelements and ash. The middle layer was conditionally usable for feeding, although even at a distance of 50 cm from the edge of the bale only partial lactic acid preservation had occurred. The centre of the bale was usable as feed. Had such bales been on the farm, at least 50% of their content would have been lost, because low-quality fodder cannot be used for feeding. Samples from Location

2 displayed similar characteristics. The undamaged bales (samples 7–9) show relatively standard parameters. By contrast, samples 10–12 again show parameters (pH levels and low lactic acid content) in their upper and middle layers that do not correspond to proper lactic acid preservation, and these samples were classified as harmful to health and not usable for feeding.Of the five mycotoxins detected in total (Table 2), only rarely in the 16 samples was an occurrence of at least one of them not observed.

Table 2. Feed analysis – mycotoxin content

sample	DON (ppb)	FUM (ppb)	AFL (ppb)	ZEA (ppb)	T2 (ppb)
1	220	15	0.3	100	50
2	220	20	0.4	120	90
3	250	20	0.1	115	80
4	1500	85	0.0	50	50
5	500	50	0.4	80	20
6	720	40	0.4	75	70
7	115	0	0.1	30	25
8	120	0	0.0	40	70
9	180	0	0.1	45	75
10	1550	200	0.15	15	60
11	980	100	0.8	20	45
12	270	0	0.4	60	65
13	170	10	0.2	15	20
14	200	10	0.0	20	20
15	220	15	0,2	27	20
16	200	20	0.0	27	20

Green mass from the first and second cuttings (samples 13–16) was contaminated at low levels with all monitored mycotoxins. This testifies to the incidence in the grass stands of producers of these substances, and also to the stability of the system and to the relative harmlessness to health from the perspective of mycotoxins in the green forage. If not fully completed, the preservation process can markedly increase the level of mycotoxins. Pathogenic organisms are present even in preserved fodder and if the fermentation process is not quickly initiated in anaerobic conditions the production of mycotoxins continues. While deoxynivalenol (DON) content in the samples from well-wrapped bales was at a low level compared with that in the green mass (samples 1–3, 7–9), in the samples from damaged bales the content was many times greater and such fodder is harmful to the health of animals. A similar situation was detected for the amount of fumonisins (FUM). Such dependence was not recorded in the amount of aflatoxins, mainly because the levels were very low. Considering the character of the producers, which are *Aspergillus* spp., such dependence is also improbable. The T-2 toxin content is interesting, as it displays greater toxic activity than that for DON, so its presence in hundreds of parts per billion serves as a warning. There presently is ongoing debate (at EC level) on defining the hygienic limit for this toxin.

Acknowledgements

Mycotoxin and chemical characteristics of silages collected from horizontal silos on farms in Co. Meath, Ireland - a pilot study

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Abstract

Mycotoxins are secondary fungal metabolites commonly found in silages and can cause a range of detrimental ailments in livestock including abortions, vomiting, lameness, immunosuppression, reduced intake or feed refusal, and reduced performance. The objectives of this study were to identify the mycotoxin challenge present in a selection of farm silages in Co. Meath, Ireland, and whether sampling position in the silo had an impact on chemical composition or mycotoxin profiles. Silages samples were collected from 2 silo locations (silage feed face vs. 3 m behind feed face) from one silo on each of 18 farms (15 grass and 3 maize). There was no difference ($P>0.05$) in pH, dry matter digestibility, lactic acid, acetic acid, water-soluble carbohydrates or crude protein concentrations between sampling locations. Mycotoxins (andrastin A, beauvericin, enniatins A[1]; B; and B[1], mycophenolic acid, roquerfortine C and zearalenone) were detected in silages, with enniatin B being the most prevalent occurring in 12 out of 36 samples (mean 0.14 mg/kg DM). Twelve mycotoxins were below the limit of detection at the feed face and the remainder were below the EU threshold. There was no effect ($P>0.05$) of sampling location on mycotoxin concentration. Samples could be taken from either silo location without affecting conventional analysis characteristics (except DM concentration) or mycotoxin concentration value.

Keywords: *Mycotoxin, silage, fungal metabolites, sampling location*

Introduction

Among the moulds identified in Irish silages, some, such as *Fusarium* and *Penicillium*, are toxigenic and thus can produce secondary metabolites named mycotoxins. The latter can induce a range of detrimental ailments in livestock including abortions, vomiting, lameness, immunosuppression, reduced intake or feed refusal, and reduced performance. In addition, consumption of animal products contaminated with mycotoxins can pose health risks to humans. Monitoring feed for mycotoxins is requested by the European Food Safety Authority and this study includes all mycotoxins that are regulated in Commission Directive (EC) No. 32/2002 and recommendation (EC) No. 576/2006. The objectives of this study were to identify the mycotoxin challenge present in a selection of farm silages in Co. Meath, Ireland, and whether the sampling position in the silo had an impact on chemical composition or mycotoxin profiles. This preliminary study was undertaken in order to identify optimal methodologies for a subsequent national survey.

Materials and methods

One horizontal silo was sampled on each of 18 farms (15 grass and 3 maize silages) during February and March 2012. Farms were randomly selected and were located within a 10 km radius of Teagasc, Grange, Co. Meath, Ireland (N 53.52, W 6.66).

Sampling

Three perpendicular core samples were taken from a 3-m wide section at the vertical silage feed face due next for removal, using a motorized corer (length 65.0 cm; internal diameter 3.5 cm). Two full depth vertical core samples were also taken from 3 m behind the silage feed face at equal distances from the silo sides, using a manual corer (internal diameter 3.0 cm). Sampling devices were thoroughly cleaned between corings. Silage samples were stored (at -20ºC) prior to subsampling for conventional chemical and mycotoxin analyses.

Conventional chemical analysis

Silage dry matter (DM) concentration was determined by oven drying (85ºC for 16 h) and was corrected for loss of volatiles according to Porter and Murray (2001). Dried (40ºC; 48 h), milled (sieve with 1 mm apertures) silage samples were assayed for *in vitro* dry matter digestibility (DMD; Tilley and Terry, 1963), water soluble carbohydrates (WSC; automated anthrone method), ash (complete combustion in muffle furnace at 550ºC for 5 h) and crude protein (N*6.25; N determined by Dumas method on a Leco FP-428 nitrogen analyser). Aqueous extracts were used to determine pH (electrode), volatile fatty acids (VFA), ethanol (gas chromatography) and lactic acid (Boehringer method).

Mycotoxin analysis

Mycotoxin analysis was carried out using an inter-laboratory validated Ultra High Performance Liquid Chromatography tandem Mass Spectrometry (UHPLC/MS^2) analytical method capable of detecting 20 mycotoxins in a single 16 minute run. This method includes regulated (aflatoxin B^1 (AFB^1), deoxynivelenol (DON), fumonisin (FUM) B^1, B^2, ochratoxin A (OTA), HT-2, T-2 and zearalenone (ZEA)) and unregulated aflatoxin B^2, G^1, and G^2, andrastin A, beauvericin, enniatin (ENN) A^1, A, B^1 & B^2, mycophenolic acid (MPA), roquerfortine (Roq) C, E) mycotoxins. The mycotoxin extraction procedure used a modified (0.1M HCl) QuEChERs platform with no clean-up step.

Statistical analysis

Conventional chemical analysis (Table 1) data were analysed using a paired t-test accounting for sampling location. Mycotoxin data (Table 2) were analysed using the Wilcoxon signed ranks test (values below the limit of detection (LOD) were censored (assigned 0.5 LOD)).

Results and discussion

There was no difference (P>0.05) in pH, DMD, lactic acid, acetic acid, WSC or crude protein concentrations between sampling locations (silage feed face vs. 3 m behind feed face). The silages sampled in this pilot study were well preserved (pH 3.85-3.89) and the proliferation of a lactic acid bacteria fermentation was evident. The higher (P<0.05) dry matter concentration at the silage feed-face than 3 m behind this may reflect (a) drying of the feed-face due to exposure to lower humidity ambient air, (b) drying of the feed face due to heat generated by respiration at the aerobic feed face and (c) moisture evaporating due to more evident frictional heating associated with the horizontal coring at the feed face than associated with vertical coring 3 m behind the feed face.

Twelve mycotoxins (aflatoxin B^1, B^2, G^1 and G^2, DON, enniatin A^1, FUM B^1, B^2, HT^2, OTA, Roq. E, T^2 toxin) were below the limit of detection at both the silage feed face and 3 m behind the feed face in all silos indicating that sampling location had no measurable impact on their concentrations. Eight mycotoxins (andrastin A, beauvericin, enniatins A^1; B; and B^1, MPA, Roq. C and zearalenone) were detected in the silages. In this study enniatin B was the most prevalent mycotoxin, occurring in 12 out of 36 samples (mean 0.14 mg/kg DM). The highest mycotoxin concentration was for MPA (1.41 mg/kg DM) and it was observed at the silage face. One EU regulated mycotoxin (zearalenone) was detected (0.076 mg/kg DM) at 4% of EU threshold. There was no effect (P>0.05) of sampling location on mycotoxin concentration. However, even though concentrations of individual mycotoxins did not give rise to concern,

caution needs to be exercised in concluding on the overall mycotoxin challenge since the effects of mixtures of mycotoxins can be more severe than the sum of their individual effects.

Table 1. Summary statistics of the conventional chemical analysis carried out on horizontal farm silages collected in Co. Meath in 2012. (** denotes $P < 0.01$)

Conventional analysis	Silage face (n=18)		3 m behind face (n=18)		
	Mean	SE	Mean	SE	*P value*
Dry matter (g/kg)	257	9.0	242	9.4	**
pH	3.85	0.111	3.89	0.086	NS
DMD (g/kg)	705	13.1	702	10.3	NS
Lactic acid (g/kg DM)	104	11.6	98	8.5	NS
Acetic acid (g.kg DM)	27	2.5	27	1.7	NS
Propionic acid (g/kg DM)	2.9	0.82	2.4	0.47	NS
Butyric acid (g/kg DM)	3.4	1.09	5.0	1.65	NS
Ethanol (g/kg DM)	16	1.9	17	1.9	NS
WSC (g/kg DM)	19	6.0	25	6.2	NS
Ash (g/kg DM)	83	8.0	83	5.4	NS
Crude protein (g/kg DM)	137	7.0	141	7.5	NS
Ammonia –N (g/kg N)	103	15.1	94	11.16	NS

Table 2. Summary statistics of the mycotoxin analysis by UHPLCMS[2] carried out on horizontal farm silages in Co. Meath in 2012. (LOD denotes Limit of Detection)

Mycotoxin	LOD	Silage face (n=18)				3 m behind face (n=18)			
		Max	Mean	SE	n >LOD	Max	Mean	SE	n >LOD
Andrastin A (µg/kg DM)	50	863	95	45.1	1	500	78.22	28.07	2
Beauvericin (µg/kg DM)	5	-	-	-	0	21.8	5.93	0.930	1
Enniatin A1 (µg/kg DM)	10	25	11	0.8	1	23.1	10.73	0.730	1
Enniatin B (µg/kg DM)	25	204	58	13.6	5	255	71.11	18.82	7
Enniatin B1 (µg/kg DM)	25	66.3	27	2.3	1	80.9	28.11	3.11	1
MPA (µg/kg DM)	40	1419	117	77.0	1	167	57.73	9.73	3
Roq. C (µg/kg DM)	40	1194	115	64.4	2	500	76.82	27.31	2
Zearalenone (µg/kg DM)	10	-	-	-	0	76.6	13.70	3.70	1

Conclusion

In this pilot survey, the challenge posed by individual mycotoxins for ruminants consuming silages was well below published guidelines. Samples could be taken from the silage feed face or from 3 m behind this without affecting conventional analysis characteristics (except DM concentration) or mycotoxin concentration values.

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Prediction of energy content of grass silages depending on grass and ensiling conditions

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Abstract

A model that predicts energy concentration of grass silages was tested on 16 farms for the years 2002 and 2004. The model is based on grass energy concentration and the ensiling conditions. Estimates of ensiling conditions depend on ensiling capacity, silo compaction, silo temperature and use of silage additives. Factors that reduced energy concentration were determined and used to derive the expected energy content of grass silage. The model was able to predict the energy content of the grass silages with a difference of 0.1 MJ NEL kg DM^{-1} and a coefficient of variation of 2.22 and 1.09%, in 2002 and 2004, respectively. For planning silage supply for different cattle groups of a holding the model delivered quality data as early as possible. In the case of farms with several silos with different silage quality characteristics, silages can be stocked best according to the special requirements of the herds. By referring the analyses to areas where the grasses originate, farmers gain valuable information for grass sward management of a particular field or pasture.

Keywords: model, sward management, feed planning

Introduction

In large agricultural farms with grassland of different sward compositions, the quality of the silages usually varies among the different silos. Knowing the grass quality, particularly the energy concentration, is a precondition for planning the effective use of the different silages according to the requirements of the different herds of a holding. In cases where there are of quality problems, a successful analysis is impossible without detailed reporting of the ensiling process.

Normally the farmer gets forage quality data that are needed for planning from the forage quality analysis of the silages. Sampling takes place by drilling and taking cores or by sampling the silo opened for feeding. Particularly in large silos, sampling of drilled cores is inefficient and does not very often supply representative results. By sampling the opened silo, results are available just at the time of feeding. There is very often a great difference in time between ensiling and feeding. Particularly in larger silos, it is nearly impossible to refer the silage analyses results to certain fields or pastures.

Materials and methods

A model named 'Normative Silokartei' was developed particularly for large agricultural enterprises at the former Institut für Futterproduktion Paulinenaue (East Germany) in the 1980s. The model predicts dry matter (DM) losses and changes in different quality parameters during the ensiling process (Weise and Rambusch, 1983). The prediction is based on forage quality analysis, evaluation of the ensilability of the ensiled grass, and on the ensiling techniques by the farmer. The model was already introduced to farmers before 1989 (Knabe *et al.*, 1986; Weise and Rambusch, 1988), but its use stopped due to the structural changes in the agricultural advisory system and, after 1989, the Northeast German farms themselves. As part of their actual grassland advisory activities the authors made use of the model in several farms since 2000 (Weise and Hertwig, 2011). In 2002 and 2004 the silage quality tool of the model was tested again under the actual practical conditions, in 5 and 9 farms respectively.

In the model, the forage quality of silage is based on the analysis of the grass forage value at time of ensiling (Figure 1).
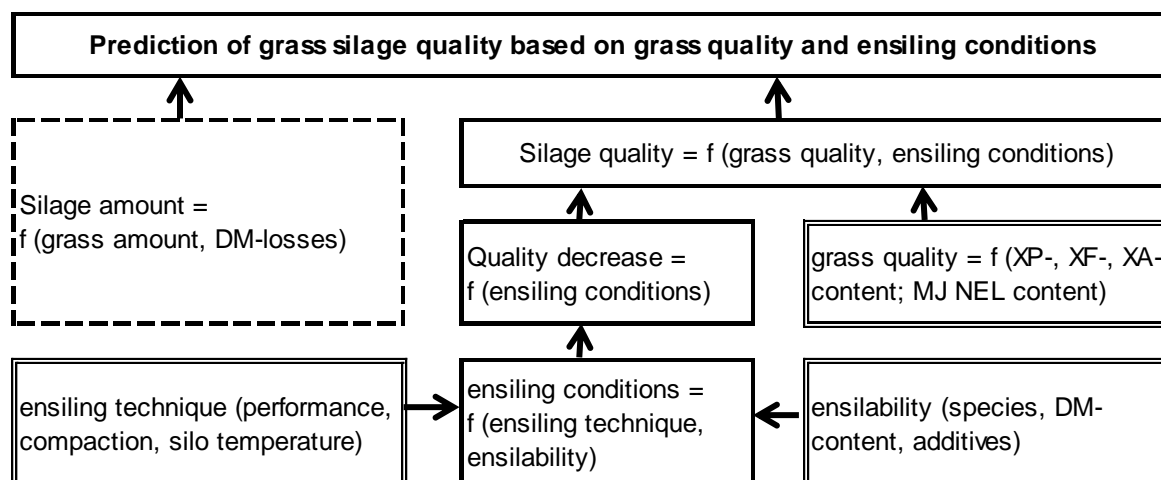

Figure 1. Structure of the model, part: prediction of silage energy content

The sampling of the grass to be ensiled and of the feed silage was carried out by the farmer throughout the entire filling and feeding time of the silo. The data used for the original model parameterization were based on the chemical analysis of forage value of the grass and the grass silage from 23 large clamp silos based on the methods and the tables from the DDR-Futterbewertungssystem (Beyer *et al.*, 1971), which provided values for crude protein (XP), crude fibre (XF), crude ash (XA) and energy concentration (EC). In the 2002 and 2004 test, forage value was estimated by near infrared (NIR)-spectroscopy (VDLUFA, 2012). Therefore the EC is given as 'Energetische Futtereinheit Rind' (EFr) per kg DM in the results of the original model in 1983 and as 'Mega Joule Net Energy Lactation' (MJ NEL) per kg DM in the test of 2002 and 2004. The 'ensiling conditions' are characterized by the 'ensilability' of the grass and the 'ensiling technique', both described by three levels: 'good', 'medium' or 'bad'. The ensilability mainly depends on the grass sward composition, the DM content at ensiling and the use of silage additives. For describing the 'ensiling technique' the silo filling und compaction process were evaluated. On the basis of a five level scale 'ensiling conditions' factors were derived to reduce grass EC ranging between 3 and 10 %.

Results and discussion

In the original 1983 model the silages contained 92% of the grass EC (Table 1).

Table 1. Energy concentration of grass, prediction and silage (all years and farms)

year	energy concentration [1]			silage/grass, %	silage/prediction, %			
	grass	prediction	silage	mean	mean	min	max	CV [2]
1983	513	484	475	92	98	91	109	4.82
2002	6.3	6.0	6.1	96	101	100	105	2.22
2004	6.4	6.1	6.2	97	101	100	103	1.09

[1] 1983: EFr kg DM[-1], 2002 and 2004: MJ NEL kg DM [-1]

[2] Coefficient of Variation

With a coefficient of variation of 4.82%, the silage EC met the predicted EC on average by 98%. In the trials of 2002 and 2004, the silages had on average 96 and 97% of the EC of the grass respectively. The coefficient of variation was 2.22% in 2002 and 1.09% in 2004, while the difference between predicted and measured silage EC was 0.1 MJ NEL kg DM[-1].
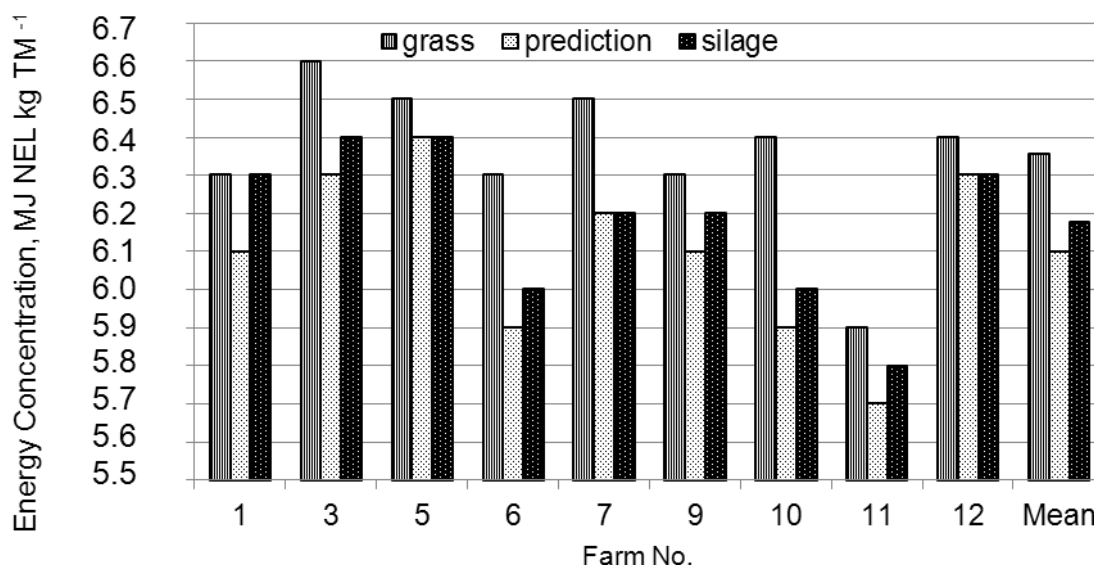
Figure 2. Energy concentration of grass, prediction and silage (all farms 2004)

Conclusions

From a practical point of view, the model successfully predicted EC of the grass silages. The important information of energy content of the silage was available early enough for planning the feeding period. Different silos, with different grass silage energy contents, could be opened and fed to the herds according to the animal requirements.

The places of origin for the different grass materials were available for the model. When the mineral content, i.e. P and K, of the samples are also available as well as the sward species composition, then the farmer has all the essential information for an effective sward management, such as fertilization, herbicide use or grass and legume reseeding (not reported).

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Predicting organic matter digestibility by two enzymatic *in vitro* methods

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Abstract

In vitro methods are regularly used to predict *in vivo* grass organic matter digestibility (OMD). To ensure accurate comparisons can be made between laboratories repeatability between methods should be checked. The objective of this study was to compare two *in vitro* methods of OMD measurement: neutral detergent cellulase digestibility (NDCFD) and pepsin cellulase (PCD60) to *in vivo* OMD. Eighteen perennial ryegrass samples were used. The samples were subsequently milled through a 1-mm screen. The OMD of the samples oven-dried at 60°C was analysed using the PCDFD method and the corresponding freeze-dried samples were analysed using the NDC60 method. The methods gave similar OMD values. The results of the two methods were then compared to *in vivo* OMD data to assess which results aligned most closely with the *in vivo* model. The *in vivo* and NDCFD OMD were significantly different while the *in vivo* and PCD60 OMD tended to be different. This study highlights the importance of comparing *in vitro* and *in vivo* methods regularly to ensure accurate OMD predictions.

Keywords: digestibility, *Lolium perenne* L., *in vitro*, *in vivo*

Introduction

Grass organic matter digestibility (OMD) is a measurement of grass quality and is a key determinant in estimating the energetic value of grass and thus the grass nutritive value. Accurate prediction of grass quality is essential to formulate diets and predict animal performance. The *in vivo* method is viewed as the 'gold standard' method for measuring OMD and is the method against which all other OMD methods are evaluated. The development of *in vitro* methods has decreased time, cost and the labour requirement for OMD measurement, while also providing accurate values (Givens and Deaville, 1999). Enzymatic methods such as the pepsin cellulase method (PCD; Aufrère and Michalet-Doreau, 1988) and neutral detergent cellulase method (NDC; Morgan, Stakelum and Dwyer, 1989) are commonly used as they dispel the need for rumen fluid by using cellulolytic enzymes to mimic digestion in the rumen. Care should be taken when comparing OMD results from different laboratories as sample preparation and the method of analysis may differ, thus altering the *in vitro* digestibility results and making comparisons difficult. The objective of this study was to compare two *in vitro* methods of OMD measurement: neutral detergent cellulase digestibility (NDC) and pepsin cellulase (PCD) to *in vivo* OMD.

Materials and methods

An *in vivo* study was conducted as a replicated 3×3 Latin square design, with three pre-grazing herbage mass treatments and three periods. A total of 18 perennial ryegrass samples were collected (one grass sample per treatment per period). Twelve wether sheep were individually housed and offered fresh grass twice-daily *ad libitum* (four sheep per treatment per period). Grass intake and faeces produced were recorded daily. A representative sample of grass offered and faeces voided from each sheep was collected daily during each period. *In vivo* OMD was determined for each treatment per period. The grass samples were dried in two ways: oven-

dried at 60°C in a Binder 720 drying oven and freeze-dried at -55°C (LS40+Chamber, MechaTech Systems Ltd., Bristol, UK). Following drying, the samples were milled through a 1-mm screen using a Cyclotech 1093 Sample Mill (Foss, DK-3400 Hillerød, Denmark). All samples were analysed for ash content by placing samples into a Gallenkamp muffle furnace size 3 (Thermo Fisher Scientific INC., Waltham, MA, USA) for 16 h at 500 °C. The crude protein (CP) concentration was analysed using a Leco N analyser (Leco FP-528; Leco Corporation, St., Joseph, MI, USA). The samples were analysed for acid detergent fibre (ADF) with an Ankom Fibre Analyser (Ankom Technology Corporation, NY, USA). The ADF values do not include ash. The grass samples oven-dried at 60°C were analysed for OMD using the *in vitro* PCD (PCD60) method of Aufrère and Michalet-Doreau (1988) as the PCD method was developed using oven-dried samples. The corresponding freeze-dried samples were analysed for OMD using the *in vitro* NDC method (NDC FD; Morgan *et al.*, 1989; Fibertec™ Systems, FOSS, Ballymount, Dublin 12, Ireland) as the NDC was developed using freeze-dried samples. *In vitro* OMD was determined on all grass samples in triplicate. PROC GLM in SAS (2002) was used compare NDCFD to PC60 and to compare the *in vitro* methods to *in vivo* OMD. Method was included as a fixed effect in the model. The Tukey Kramer multiple range test was used for mean separation (*P* < 0.05).

Results and discussion

In vivo OMD was within the range reported for perennial ryegrass. The CP concentration of the grass samples ranged from 127 to 303 g kg⁻¹ (mean = 216 ± 47.9 g kg⁻¹). The ADF concentration ranged from 217 to 285 g kg⁻¹ (mean = 256 ± 19.1 g kg⁻¹). The ash content ranged from 61.7 to 71.9 (mean = 72 ± 8.2 g kg⁻¹). The chemical composition of the grass was within the ranges reported for predominately perennial ryegrass grazing swards (O'Neill *et al.*, 2013). Both *in vitro* OMD methods gave similar grass *in vitro* OMD values (*P*>0.05). The results of the two methods were then compared to *in vivo* OMD data to assess if the results aligned closely with the *in vivo* model (Figure 1).

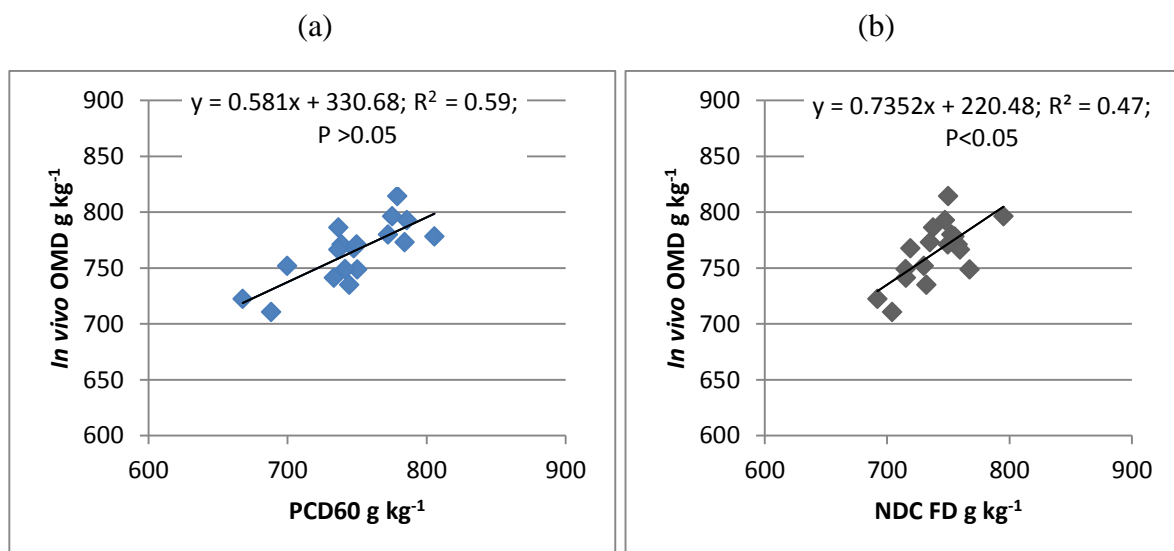(a)   (b)

Figure 1. The relationship between perennial ryegrass *in vivo* organic matter digestibility (OMD; g kg⁻¹) and OMD predicted by two *in vitro* methods: (a) pepsin cellulase method (PCD60) using samples oven-dried at 60°C and (b) neutral detergent cellulase method (NDC FD) using freeze-dried samples

A regression equation is necessary to relate *in vitro* OMD values to the *in vivo* method allowing for the *in vivo* OMD of future samples to be derived from the *in vitro* result. The regression equation currently used for the NDCFD method was developed in the 1980s using the *in vivo*

OMD of dairy cows and since then the equation has not be evaluated (Morgan *et al.*, 1989). Since the 1980s, there have been significant improvements in grass breeding, grass management and animal genetics. These improvements may not be reflected in the regression equation being used to relate NDCFD OMD data to *in vivo* OMD. Regular validation of any method is essential to ensure its accuracy over time. Unlike the NDC, which had a once-off evaluation, the PCD60 method is regularly evaluated (Aufrère *et al.*, 2007). Aufrère *et al.* (2007) developed plant species-specific equations to predict OMD and the equation used for perennial ryegrass also incorporates permanent pasture. The chemical composition of the grasses used by Aufrère *et al.* (2007) differed to the chemical composition of the grass used in the present study, which may explain why the PCD60 tended to predict different OMD compared to the *in vivo* OMD. In that study CP concentration ranged from 69 to 266 g kg^{-1} (mean = 150 g kg^{-1}), ash content ranged from 55 to 193 g kg^{-1} (mean = 110 g kg^{-1}) and crude fibre concentration ranged from 186 to 405 g kg^{-1} (mean = 263 g kg^{-1}), which equates to a mean ADF concentration of 287 g kg^{-1}. Despite this difference in chemical composition the PCD60 method was able to accurately predict *in vivo* OMD.

Conclusion

The PCD60 and NDCFD methods gave similar OMD results. The *in vivo* method provides the benchmark against which all other methods of measuring OMD are evaluated. Compared with *in vivo* OMD, the NDCFD method gave significantly different OMD results while the PCD method tended to give different OMD results. The PCD60 method is regularly evaluated and was able to predict *in vivo* OMD more accurately than the NDCFD method. This study highlights the importance of comparing *in vitro* and *in vivo* methods regularly to ensure accurate OMD predictions.

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Carbon sequestration in silage maize as affected by N fertilization

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Abstract

Changes in soil organic carbon (SOC) stocks of forage cropping systems have a large impact on their greenhouse gas balance. Analysis of a long-term silage maize experiment indicate a positive impact of N fertilization on SOC stocks. Observed differences among treatments were higher than values obtained from a carbon balance based on literature data.

Keywords: soil organic carbon, maize, yield, N fertilization, carbon balance

Introduction

Reducing the carbon footprint (CF) along the production chain, especially in forage production, which contributes the major share of greenhouse gas emission, is regarded a key to reduce 'Livestock's Long Shadow'. Knowledge of mechanisms governing C sequestration, which has a significant impact on the CF of forage crops, however, is still limited. The objectives of the current study were to investigate the impact of N fertilization on SOC stocks in silage maize, based on a long-term experiment.

Materials and methods

The study was based on a continuous silage maize experiment with four replicates, established in 1993 on a sandy sand soil (pH 4.8-5.3) in northern Germany. Nitrogen fertilization was varied in seven levels: control (0 kg N ha^{-1}), three mineral N treatments (30, 70, 110 kg N ha^{-1} applied as calcium ammonium nitrate (CAN)), and three cattle slurry (CS) treatments (20, 30, 40 m³). All fertilized treatments received a banded N starter, 40 kg N ha^{-1}. Further nutrients were applied according to good agricultural practice in order to avoid any nutrient deficiencies. After 18 years of varied N supply, samples for SOC determination were taken in autumn 2011 in three soil layers (0-30, 30-60 and 60-90 cm). Samples were dried, sieved (< 2 mm), and ground to fine powder. Total C and N contents were measured using a CN-analyser (Vario Max CN, Elementar Analysensysteme, Hanau, Germany). The organic C content was obtained as the difference of total C and carbonate content (Scheibler, DIN ISO 10693). Soil bulk density was determined at 10-15, 40-45, and 75-80 cm depth, applying a core method. The belowground data were supplemented by an analysis of dry matter (DM) yield. A one-factorial analysis of variance was conducted to investigate the effect of N fertilization on SOC stocks, using SAS 9.2 Proc mixed. The impact of N fertilization on maize yield was analysed by first fitting a three-parameter exponential function to each combination of N treatment and replicate and then conducting one-factorial analyses of variance for each parameter separately. Multiple comparisons of means were conducted by the Tukey-Kramer method.

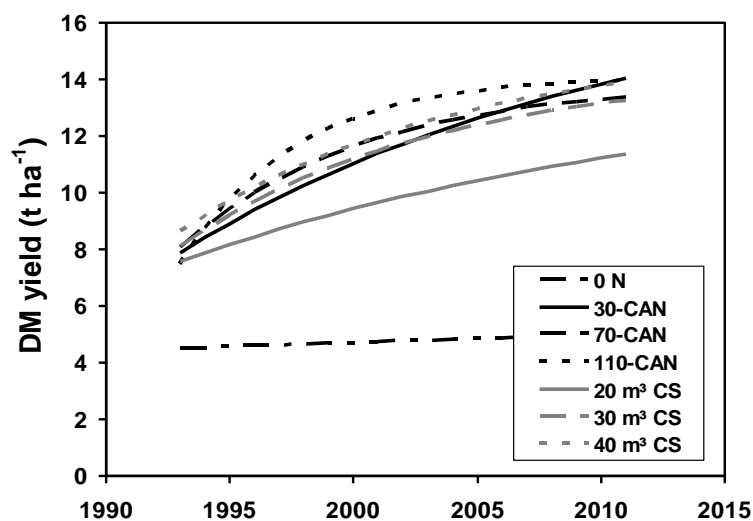## Results and discussion

Figure 1. DM yield response functions as affected by N fertilization.

Over the 18-year experimental period an increase of DM yield was found for all fertilized treatments, probably due to residual N effects from fertilizer and maize roots, while yield of the control did not change over time (Figure 1). Thus, the control exhibited a significantly different response function to the remaining treatments. In addition, significant differences were detected between 110-CAN and all slurry treatments, as well as between 30-CAN and 110-CAN. Soil bulk density tended to be lower for the 0-N and 70-CAN treatments, but no significant impact was found (not shown). Soil organic C stocks were highest in the top layer, but still considerable amounts were detected in the subsoil (Table 1). It is known from literature that often more than 50% of the SOC stock is found in deep soil horizons (Batjes, 1996), affected by root depth allocation, leaching, abiotic decay conditions, and bioturbation. SOC stocks were higher in the fertilized treatments compared with the control in the 0-30 cm layer, but not in the subsoil. A significant impact of N fertilisation became evident only in the lowest soil layer, where the 30 m³ CS treatment revealed up to 30 t ha^{-1} higher SOC content than 0-N, 70-CAN and 20 m³ CS.

Table 1. Soil organic C stocks (t C ha^{-1}) as affected by N fertilization treatment and soil depth.

Soil depth	Nitrogen treatment						
	0-N	30-CAN	70-CAN	110-CAN	20 m³ CS	30 m³ CS	40 m³ CS
0-30 cm	78.5	92.0	89.7	85.3	91.9	96.4	99.2
30-60 cm	52.0	59.9	49.0	49.0	47.1	57.2	56.0
60-90 cm	20.0[a]	35.7[ab]	23.0[a]	27.7[ab]	21.9[a]	50.1[b]	28.1[ab]

Although mostly not significant, we evaluated if the differences among treatments could be reproduced when applying a C balance approach. To this end, the C remaining in the soil from the C input in terms of slurry, maize roots and stubble was estimated based on literature values. In detail, we assumed:

- a stubble-to-shoot ratio of 6% - 8%, with shoot DM yield taken from measurements
- a root-to-shoot ratio of 0.2-0.28 (depending on N fertilization; Amos and Walters, 2006)
- a stubble and root C content of 44%
- a ratio of C-rhizodeposition to total root C input of 0.30-0.44 (depending on N fertilization; Amos and Walters, 2006)
- a root turnover of 11% (Hullugalle et al., 2010)
- a method-related root underestimation of 36% (Subedi et al., 2006)
- 31% of the C input (root, stubble) and 26% of the slurry-C to remain in soil (Flessa et al., 2000; Bertora et al., 2009)

The estimated C amounts remaining in soil for the different treatments ranged between 8 and 20 t C ha^{-1} and highly correlated with the corresponding measured soil organic C stocks (R = 0.87*) in the top soil layer, while the relationship was less close (R = 0.68*) for the 0-60 cm layer. When comparing the differences in calculated C remaining and the measured C stocks among the treatments, we found similar agreement for the topsoil and the 0-60 cm layer. However, the observed differences among treatments were considerably larger than the estimated C remaining in soil from slurry and maize C input, as indicated by an intercept and a slope significantly different from zero and unity, respectively (Figure 1). The assumptions taken in our C balance calculation thus considerably underestimate either the C input from maize
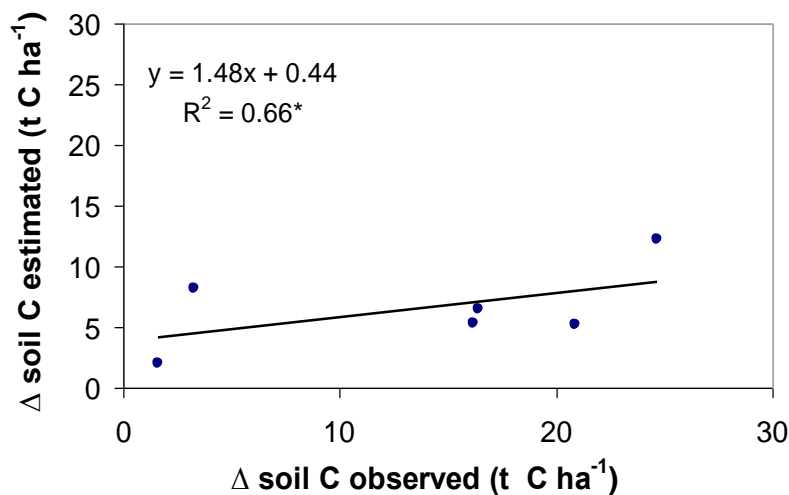

and/or slurry or overestimate their decomposition rates. The root-to-shoot ratio is a key factor determining the C input. A ratio of 0.2 at silage maize harvest was estimated in a meta-analysis by Amos and Walters (2006). The underlying data, however, revealed a large variation and were based largely on older studies. Recent results by Qi *et al*. (2012) indicate that yield progress can partly be attributed to an increased root-to-shoot ratio.

Figure 1. Observed differences in soil C stocks among treatments of the 0-60 cm layer (t C ha^{-1}) versus corresponding calculated carbon remaining from slurry and maize C input over the 18-yr period, with treatment 40 m³ CS serving as reference.

Conclusion

Observed and calculated differences in SOC stocks among N treatments reveal further need for research with respect to soil C changes in silage maize production. Future work will therefore focus on the application of models to investigate the processes governing carbon fluxes in silage maize cultivation.

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Accuracy of forage intake estimation with three different indirect prediction models

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Abstract

The aim of the study was to compare the precision of the dry matter (DM) intake prediction among three different indirect methods. Observed DM intake from two trials carried out with two groups of 15 and 12 cows fed on TMR *ad libitum* containing maize silage mixed with *Vicia faba* silage or grass hay, under continuous stall conditions. Dry matter intake was measured by using a computerized system proposed by Bach *et al.* (2004). The results of measured DM intake were compared with three indirect methods for estimating DM intake: a) intake prediction equations of NRC (2001); b) the animal performance method (APM) proposed by Macoon *et al.* (2003), and c) the model of prediction of intake capacity (IC; Faverdin *et al.*, 2011). The actual DM intake was 20.19 kg d^{-1} and the estimated intakes were 21.60, 18.30 and 24.07 kg DM d^{-1} for NRC, APM and IC methods respectively. Considering the standard error of prediction, the APM method performs better in relation to the actual intake. Using the APM model, the correlation between the estimated value and the actual value was better when the model is applied to dairy cows with a milk production higher than the average production ($R^2=0.85$). The APM method has 95% prediction accuracy, and can be used as a non-invasive tool that affects animal´s DM intake.

Keywords: dairy cow, feed intake, estimation methods

Introduction

Milk production based on grazing represents an opportunity to reduce production costs and improving farm sustainability. However, accounting for forage intake is one of the most difficult tasks. Several methods have been developed in order to estimate forage intake, and the use of markers like N-alkanes or Cr are among the most frequently used. However, they are laborious, time consuming and expensive; therefore, they are unviable in the current context of funding crisis in scientific research. Other tools allow us to make forage intake estimations indirectly, by using prediction equations based on animal´s performance, physiological status and diet quality. The aim of this study was to determine the precision of forage intake prediction by comparing different methods against actual forage dry matter (DM) intake, in order to validate estimates applied to grazing animals.

Materials and methods

Measurements were made of DM intake from two trials carried out with two groups of 15 and 12 cows fed on TMR *ad libitum* containing maize silage mixed with *Vicia faba* (trial 1) or grass hay (trial 2), under continuous stall conditions. During each assay, the DM intake of individual cows was automatically recorded by an electronic weighing system integrated to the scale pans using a computerized system (Bach *et al.*, 2004) in three periods of seven days each. Milk yield was recorded and sampled daily at both milking times from each cow. All cows were weighed and body condition score recorded on the first and last day of each period in both assays after the morning milking. Nutritive values of both TMR were analysed by NIRS. Milk samples were

analysed by a Milko Scan FT6000. The details of these assays are described in Morales-Almaráz *et al.* (2010). The results of measured DM intake were compared with three indirect methods for estimating DM intake: a) intake prediction equations of NRC (2001), based on the energy requirements of animals; b) the animal performance method (APM) proposed by Macoon *et al.* (2003), which includes net energy content of feeds, and dairy cow requirements for maintenance, lactation and physical motion, and c) the model of prediction of intake capacity (Faverdin *et al.*, 2011), based on three factors that affect the ability of intake (IC): body weight, potential milk production modified by the metabolizable protein, and body condition. Comparison of means was performed using the Paired-Samples t-test procedure.

Results and discussion

The actual intake of dry matter was 20.19 kg d^{-1} and the estimated intake was 21.60, 18.30 and 24.07 kg DM d^{-1} for NRC, APM and IC methods respectively. APM methods underestimated DM intake in 5%, whereas IC and NRC overestimated in 26 and 13% respectively (Figure 1). Considering standard error, the APM performs better, being highly related to real estimations of DM intake, whereas IC is the less accurate, as this method estimates animal´s maximum DM intake. The higher accuracy of the APM method can be explained because is directly related to the animal´s performance, and only integrates individual intake per animal, but its disadvantage is that intake response is only for a defined period of time.
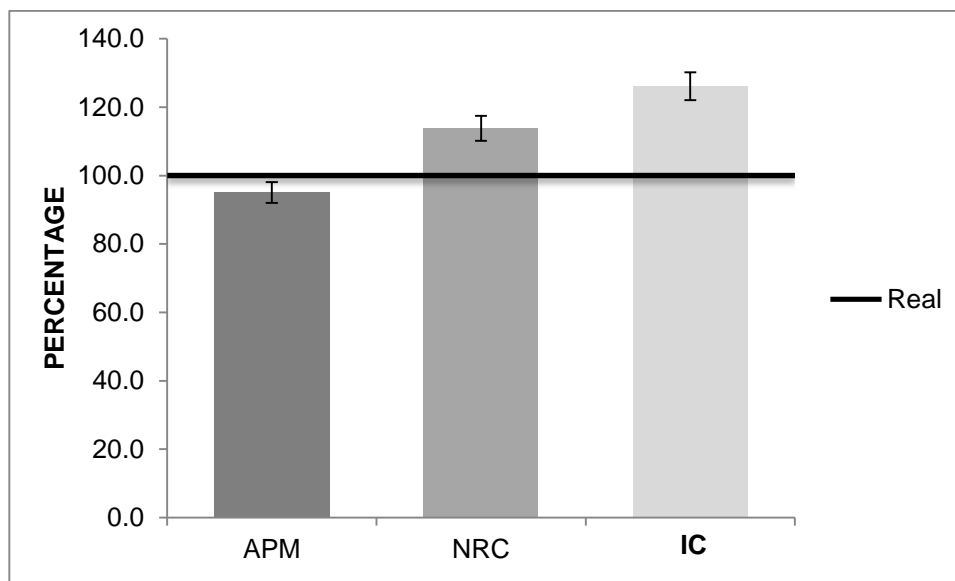

Figure 1. Accuracy in the estimation of dry matter intake (as percentage, actual intake was 100%). [APM: Macoon *et al.*, 2003; NRC: NRC, 2001; IC: Faverdin *et al.*, 2011].

Considering that APM is the more accurate prediction method, a correlation between this method and milk production was determined. For above-average milk yields (35 L d^{-1}), the APM predictions are more accurate ($R^2=0.85$), than for below-average yields ($R^2=0.56$; Figure 2).
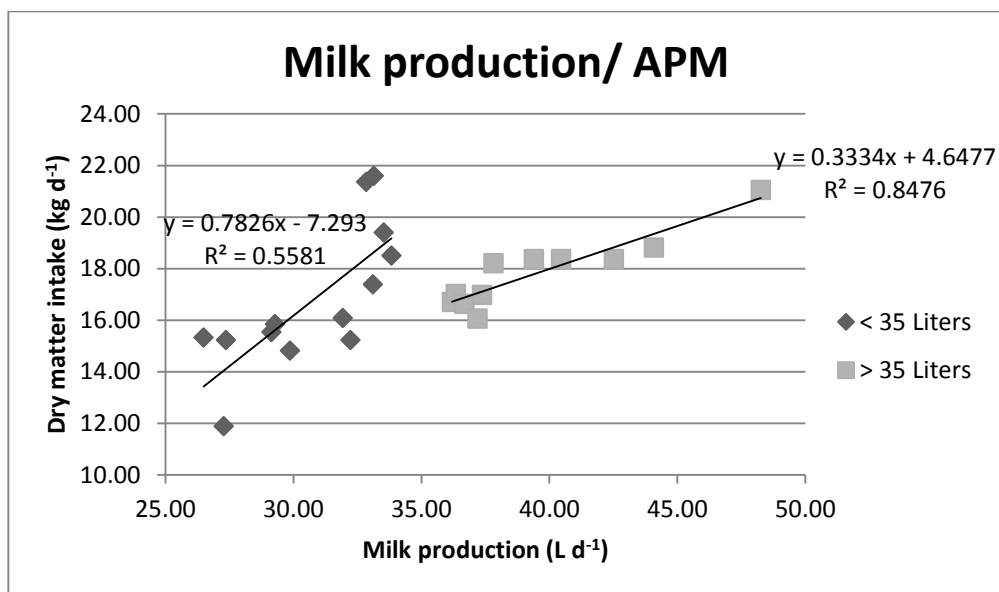
Figure 2. Correlation between milk yields and APM forage intake prediction

Conclusions

The APM is the most accurate indirect method for dry matter estimation, with predictions up 95% related with the actual measured intake, and it can be used as a non-invasive tool without affecting the animals' forage intake.

Acknowledgements

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Compatibility of using TiO₂ and the faecal near-infrared reflectance spectrometry for estimation of cattle intake

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Abstract

Combining titanium dioxide (TiO₂) as indigestible marker to faecal near-infrared reflectance spectrometry (F-NIRS) can be used to determine cattle feed intake and quality of ingested forage if F-NIRS spectra are not modified by the marker. This study aimed at determining the compatibility of TiO₂ with F-NIRS. Three dry cows were fed a standard hay-based diet for three weeks supplemented with a daily dose of 0.1% (10g) TiO₂ during the last two weeks of the experiment. Faeces samples were collected every day and analysed for TiO₂ and F-NIRS. Results suggest that TiO₂ did not interfere with F-NIRS analyses. The calculations of crude protein, NDF, ADL contents, as well as dry matter intake did not change over time with increasing TiO₂ in the faeces ($P > 0.05$). Slight differences observed for other predicted parameters seemed to be independent from TiO₂. The higher Mahalanobis distance (H) for chemical composition ($H = 7.2$) independent from TiO₂ inclusion could indicate that faecal spectra did not correspond exactly to the prediction database. Although 0.1% incorporation of TiO₂ seem not to interfere with F-NIRS measurements, caution must be taken with higher levels of TiO₂ as nothing indicates that interference could not appear.

Keywords: Ruminant, titanium dioxide, faecal near-infrared spectrometry, intake, diet chemical composition.

Introduction

Methods used to determine feed intake and quality of consumed forage in grazing cattle are time-consuming, expensive and sometimes controversial in respect to animal welfare as they include different techniques such as sward clipping techniques or oesophageal fistulated animals (Decruyenaere *et al*., 2009). A combination of an indigestible marker to faecal near-infrared reflectance spectrometry (F-NIRS) can provide a useful alternative, providing that the marker fed daily to the animal does not interfere with F-NIRS spectra (Titgemeyer *et al.*, 2001; Decruyenaere *et al., 2012*). As previous studies report that Cr₂O₃ is likely to interfere with NIRS calibration data (Decruyenaere *et al.*, 2012), this study investigates if titanium dioxide (TiO₂) used as indigestible marker was compatible with F-NIRS analysis.

Materials and methods

A three-week experiment was performed on three dry red-pied cows housed in free stalls in the Animal Science Unit of GxABT (Gembloux, Belgium). All cows received 7 kg d⁻¹ of standard temperate hay and 2 kg d⁻¹ of a mixed concentrate and had a free access to water. After an adaptation period of one week, 10 g of TiO₂ mixed with 50 ml of molasses was distributed every day to each cow until the end of the experiment. The faeces were collected every day, dried at 60 °C, and ground to pass a 1-mm screen prior to TiO₂ and F-NIRS analyses. TiO₂

dosage in faeces was performed according Myers *et al.* (2004). The F-NIRS analyses were achieved as described by Decruyenaere *et al.* (2012). The chemical composition, dry matter intake (DMI) and *in vivo* organic matter digestibility (OMD) predicted from the F-NIRS database were compared daily along the entire experiment using the MIXED procedure of SAS 9.2 with the 'cow×day' as experimental unit. The correlation between these parameters and TiO_2 content in the faeces was calculated with the CORR procedure of SAS 9.2.

Results and discussion

Figure 1 shows the evolution of TiO_2 contents in the faeces before and during the daily incorporation of 10 g of TiO_2 in the diet (day 6 being the first day of TiO_2 distribution). During the adaptation period without TiO_2, its faecal content was equal or close to zero, before increasing and then reaching a plateau towards the end of the experiment; so the TiO_2 dosage did not face interference problems. Dietary TiO_2 did not interfere with the F-NIRS analysis.
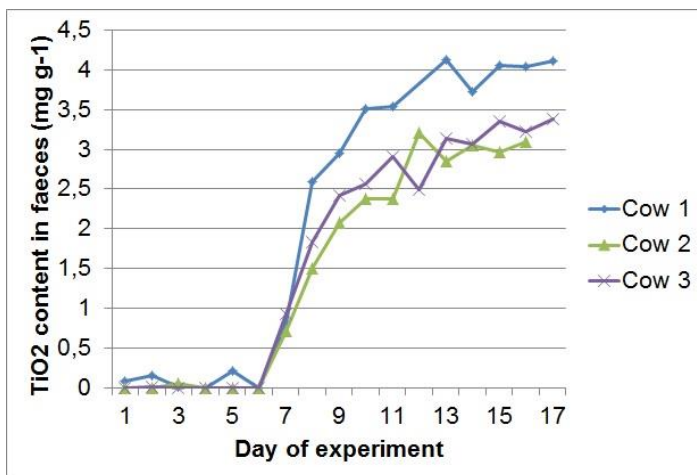

Figure 1 Evolution of TiO_2 contents (mg g^{-1}) in the faeces of the three cows

Prediction of crude protein (CP), neutral detergent fibre (NDF) and acid detergent lignin (ADL) contents as well as the DMI (Figure 2) did not change over time ($P > 0.05$; $P = 0.0723$ for NDF content as the lowest P-value). Despite some changes along days for the acid detergent fibre (ADF, $P = 0.0381$) content and the *in vivo* OMD ($P = 0.0009$) (Figure 2), the slightly different values seemed to appear independently before or after ingestion of titanium dioxide. The DMI ($P = 0.4613$; Figure 2), which seemed more fluctuating along the experiment, could be explained by individual differences of intake; for example, the 13th day of the experiment, the DMI of one cow reached 85.3 *vs* 47.5g kg^{-1} metabolic weight (MW) for that of another cow. The standardized Mahalanobis distance (H) which evaluates the correspondence between the faeces spectra and the F-NIRS database should ideally be lower than 3 for an accurate prediction (Shenk and Westerhaus, 1991). For OMD and DMI, the average distance H was below 3 for the DMI and the OMD ($H = 2.8$ and 2.87 respectively; two thirds of the samples being lower than 3) while it reached 7.2 for the chemical composition. This should probably not be due to TiO_2 inclusion in the diet, but rather to a discrepancy between the samples and the calibration dataset.

The results of correlation between the titanium dioxide content in the faeces and the parameters predicted by F-NIRS should be considered with caution. Indeed, most of parameters, as DMI or OMD, were not significatively correlated to the TiO_2 content ($P > 0.05$; $P = 0.1086$ and $r = 0.23206$ for CP as the highest P-value and correlation coefficient (r)) but other parameters were significatively correlated to TiO_2 content, such as total ash (TA), NDF and ADF content ($P < 0.0001$ and $r = 0.58651$ for TA).
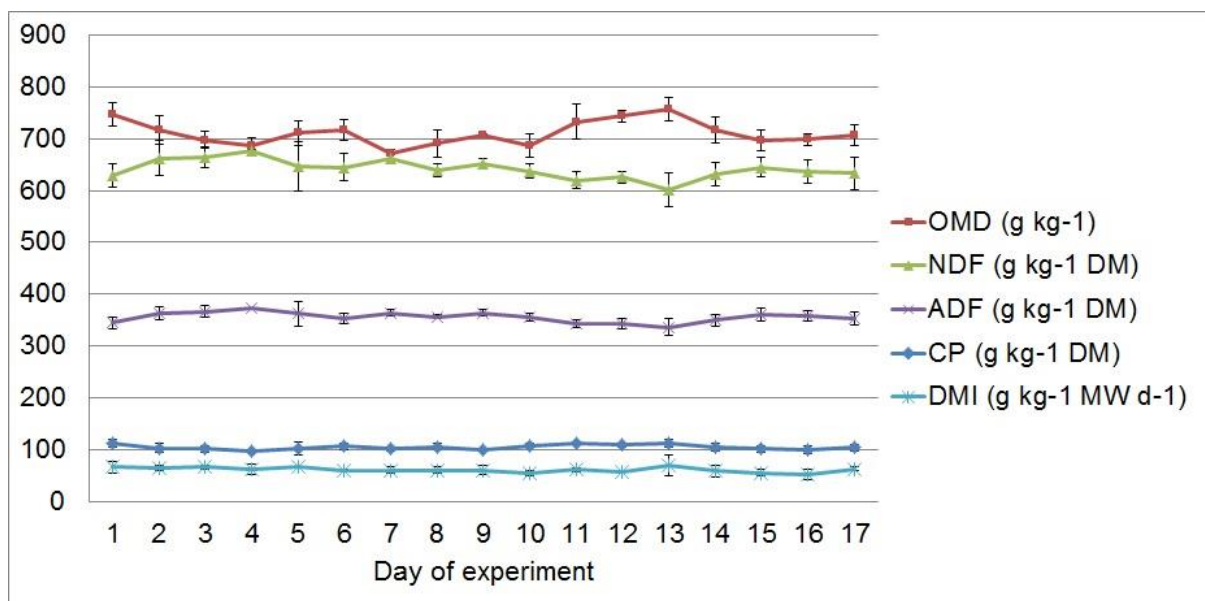
Figure 2: Means and standard deviation of CP, NDF and ADF contents (g kg^{-1} of DM) of the diet and the DMI (g kg-1 MW d-1) and *in vivo* OMD (g kg^{-1}) predicted by F-NIRS along the experiment

Conclusion

Feeding 10 g d^{-1} TiO$_2$ as indigestible marker in cattle (0.1% incorporation level) did not interfere with F-NIRS prediction. The use of these results should be done with caution, as there is nothing that indicates that interference could not appear when higher levels of TiO$_2$ are incorporated in the diets.

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Dry matter intake and *in vivo* digestibility of four perennial ryegrass cultivars

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Abstract

There is renewed interest in improving animal performance in grazing systems by using highly productive perennial ryegrass cultivars. Four cultivars (Glenroyal (GR), Delphin (DE), Tyrella (TY) and Astonenergy (AS)) were evaluated for *in vivo* dry matter intake (DMI) and dry matter digestibility (DMD) using Texel wether sheep housed in digestibility stalls. The structural profile and leaf, stem, and dead proportion of the sward were also measured. There was no effect of cultivar on DMI during two time periods (May/June (LS1) 1.53 ± 0.069kg and Aug/Sept (LS2) 1.50 ± 0.065 kg ($P>0.05$)). There was a cultivar $\times$ LS interaction for DMD. In LS1, TY had a greater DMD than DE ($P<0.05$). A reduction occurred in DMD from LS1 to LS2. This was associated with an increased amount of dead material in the sward in LS2. In LS2 there was no significant difference between cultivars in DMD but TY and GR had a greater proportion of dead material than DE and AS. These results indicate that there are small differences between perennial ryegrass cultivars in DMD but large effects of season on perennial ryegrass cultivar DMD.

Keywords: *In vivo* digestibility, dry matter intake, perennial ryegrass, cultivar

Introduction

Dairy systems that maximize grass utilization are highly competitive and sustainable (Peyraud *et al.*, 2010). However, fundamental to maintaining this competiveness is increasing efficiency within the system, by using new innovations and technology. Reseeding permanent pasture with perennial ryegrass *(Lolium perenne* L.) has been shown to increase farm profitability (Shalloo *et al.,* 2011). Perennial ryegrass is the most commonly sown grass species in temperate climates as it has high growth potential and high nutrient value (Tas *et al*., 2005). Cultivars of perennial ryegrass vary in feeding value, due to differences in ploidy and heading date (Gowen *et al.*, 2003) as well as sward structure (Wims *et al*., 2013). Tetraploid cultivars have higher *in vitro* organic matter digestibility (OMD) than diploid cultivars (Wims *et al*., 2013). There is a need to quantify any differences between cultivars *in vivo*, to be confident that *in vitro* differences are truly representative. The objective of this experiment was to determine the effect of four perennial ryegrass cultivars on dry matter intake (DMI) and dry matter digestibility (DMD) in sheep.

Materials and methods

Twelve Texel wether sheep were used to determine the DMI and *in vivo* DMD of four perennial ryegrass cultivars. The experiment was run as a 4×2 incomplete Latin square design. The four treatments (TRT) comprised four perennial ryegrass cultivars: Astonenergy (tetraploid, heading date 31 May) (AS), Delphin (tetraploid, heading date 1 June) (DE), Tyrella (diploid, heading date 3 June) (TY) and Glenroyal (diploid, heading date 3 June) (GR). The experiment was repeated twice: the first Latin square (LS1) was from 13 May to 7 June 2013 and the second Latin square (LS2) was from 19 August to 13 September 2013. All cultivars were sown in spring 2012 at a sowing rate of 32 kg/ha for diploids and 40 kg/ha for tetraploids. In April 2013, six experimental plots per cultivar were marked and each plot was designed to feed 3 sheep for

4 d. These plots were cut 3 to 4 weeks prior to feeding, to allow a pre-grazing herbage mass of approximately 1500 kg DM/ha at the time of feeding. The sheep were housed in individual stalls allowing for the total collection of urine and faeces. Body weight was a blocking factor at the start of each LS. Each LS had two periods and each period consisted of 12 days: 6 days adaptation and 6 days measurement (MP). Fresh grass was cut and chopped daily using a motor Etesia (Etesia UK Ltd., Warwick, UK). Sheep were fed *ad libitum* (110% of DMI) and grass DMI was recorded daily. Feeding times were 0900h and 1630h. During the MP, a representative sample of the grass offered and faeces voided by each sheep was collected daily. Daily grass and faeces samples were dried and then bulked to give one sample of each per cultivar per MP. On day-8 of each period a 40 g sample of each cultivar was separated into leaf, pseudostem, true stem and dead proportions >4 cm. The sward profile was measured from ground level prior to cutting once during each MP. The extended tiller height (ETH), pseudostem height (PH) and free leaf lamina (FLL) were measured on 100 tillers. Pre-grazing herbage mass was measured using Gardena hand shears (Accu 60, Gardena Int. GmbH, Ulm, Germany) and a 0.25 m^2 quadrat, four times per cultivar during each period. Data for DMI, DMD and leaf, pseudostem, true stem and dead proportions >4 cm were analysed using PROC MIXED in SAS (2002). Treatment, period within LS, LS and the interaction between LS and treatment were included as fixed effects. Sheep was included as the random effect. Sward structural effects were analysed using PROC GLM and treatment, period within LS, LS and the interaction between LS and treatment were included as fixed effects.

Results and discussion

Results are presented in Table 1.

Table 1. Dry matter intake (DMI), *in vivo* digestibility (DMD), leaf, stem, dead proportions, and sward profile of cultivars: Astonenergy (AS), Delphin (DE), Tyrella (TY) and Glenroyal (GR)

	TRT	DMI (kg DM)	DMD (g/kg)	Leaf (%)	Pseudo-stem (%)	True stem %	Dead (%)	ETH (mm)	PH (mm)	FLL (mm)
LS1	AS	1.44	837[ab]	62.9[ac]	21.0[b]	6.9[a]	9.3[a]	256.2	105.5	150.6
	DE	1.53	796[b]	63.5[abc]	23.3[a]	5.5[b]	8.1[a]	243.6	91.2	153.0
	TY	1.58	845[a]	63.3[abc]	24.4[a]	3.7[c]	8.7[a]	225.8	98.6	127.1
	GR	1.58	826[ab]	65.2[abc]	21.0[b]	4.7[cb]	9.1[a]	191.7	70.7	121.5
LS2	AS	1.57	734[c]	67.9[ab]	15.9[c]	0	15.9[b]	302.7	65.3	237.5
	DE	1.59	746[c]	68.1[b]	17.2[c]	0	14.9[b]	295.5	66.0	229.5
	TY	1.48	717[c]	64.3[abc]	15.7[c]	0	19.1[c]	293.6	60.3	233.0
	GR	1.38	713[c]	61.6[c]	15.5[c]	0	21.9[c]	243.8	53.0	190.9
SEM		0.115	15.4	1.45	0.67	0.48	1.08	14.21	6.85	18.6
TRT		NS	*	*	***	**	***	*	NS	NS
LS		NS	***	NS	***	n/a	***	*	**	*
LS× TRT		NS	**	***	***	n/a	***	NS	NS	NS

ETH-Extended tiller height, PH- pseudostem height, FLL, free leaf lamina,

For DMD, there was a significant treatment × LS interaction *(P<0.01)*. In LS1, TY had a significantly higher DMD than DE *(P<0.05)*. The other two cultivars were intermediate

(P>0.05). In LS2, all four cultivars had similar DMD to each other (P>0.05). The difference in DMD between cultivars in LS1 could be due to differences in chemical composition (Wims *et al*., 2013) or to differences in sward structure (Beecher *et al*., 2013) which can be particularly evident during the reproductive phase. The reproductive phase coincided with LS1. In LS1 there was a higher amount of true stem in DE compared to TY, which agrees with the lower DMD found in DE. True stem has been shown to reduce the digestibility of swards (Beecher *et al*., 2013). The DMD of all cultivars decreased significantly from LS1 to LS2. The decrease in DMD in LS2 compared to LS1 may be attributed to a high soil moisture deficit in August and September 2013. This resulted in an increase in the dead proportion in the sward, which is the most indigestible morphological fraction in perennial ryegrass (Beecher *et al*., 2013). The dead proportion was higher in LS2 compared to LS1 (P<0.001) for each cultivar. This also agrees with (Hazard *et al*., 1998) who found that differences in digestibility between cultivars were associated with differences in the proportion of dead material in the top lamina. The PH was significantly higher in LS1 P<0.01), this shows that the stem was lengthening as it was becoming reproductive, and agrees with the higher proportion of true stem in LS1. This, combined with a lower ETH in LS1 (P<0.05), lead to a lower FLL content in the sward in LS1 than in LS2 (P<0.01). The lower FLL in LS1 did not however lead to lower DMD.

The DMI for LS1 was 1.53±0.069 kg and for LS2 was 1.50 ±0.065 kg (P>0.05). There was no difference between cultivars for DMI during both LS. This differs from (Gowen *et al*., 2003) (O'Donovan and Delaby, 2005) who found that cows differed in their intake of cultivars, but in those studies the animals were grazing. In the present study the grass was cut and offered to the animals indoors. When cultivars were cut and fed to animals inside there was no difference in DMI (Tas *et al*., 2005), similar to the current study.

Conclusion

This study shows that cultivar has a small effect on sward DMD but season had a large effect on sward DMD. In order to improve the nutritive value of a sward, it is necessary to minimize the dead proportion of the sward.

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Accurate monitoring of the rumination behaviour of cattle using IMU signals from a mobile device

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Abstract

Improving the monitoring of rumination in cattle could help in assessing their welfare status and their risk of acidosis. In this work, the monitoring of cattle behaviour was performed using the inertial measurement unit (IMU) present in smartphones mounted on the neck of cows. The processing of both time and frequency domains of the IMU signals was capable of detecting accurately the main behaviours (grazing, rumination and other) and highlight the characteristics of the rumination process. The algorithm for analysis of rumination was more accurate for grazing cattle than for silage-fed cattle in stables.

Keywords: cattle, behaviour, rumination, signal processing, IMU

Introduction

All animal species display behaviours that indicate their physical, physiological and welfare status (Frost *et al.*, 1997). For ruminants, grazing, ruminating and resting behaviours occupy more than 90% of the time budget of the animal on pasture (Kilgour, 2012). Rumination represents 5 to 9h/d for cattle (Vallentine, 2001). It is a cyclic process which completes the chewing of fibrous ingested feed after it has undergone anaerobic fermentation by microbes in the rumen. A cycle begins with the regurgitation of a rumino-reticular bolus followed by semi-circular jaw movements and ends with the deglutition (Jarrige *et al.*, 1995). Rumination routines are influenced by pasture quality, intake quantity especially in fibrous content (Jarrige *et al.*, 1995; Fustini *et al.*, 2011), physiological status, and the level of stress and anxiety of the animal (Soriani *et al.*, 2012; Braun *et al.*, 2013). In dairy cows, high-yielding individuals are fed high levels of concentrates leading to a low level of insoluble fibre intake. This low level of fibre puts them at risk of both acute and chronic acidosis, since it induces a decrease in the duration of the rumination in the daily cycle (De Vries *et al.*, 2009). Characterizing rumination is therefore an interesting indicator of health and welfare in ruminants.

Recent developments in sensor technology lead to their possible use for automated detection of domestic ruminants' main behaviours, such as rumination or grazing using mainly GPS and accelerometers (Swain *et al.*, 2008). The present work aimed at developing a white-box approach to detect differences in rumination patterns in order to enable its accurate monitoring based on signal processing of a mobile device's IMU fixed on cows.

Material and methods

Two red-pied dry cows were fitted with an iPod Touch 4G or an iPhone 4S on their neck. Nine recording sessions were performed between September 2012 and November 2013: seven with the cows grazing a ryegrass-white clover pasture, and two in stables when cows were fed silage-based diets. Each session included: (1) 100Hz data acquisition from the 3-D accelerometer and the 3-D gyroscope of the IMU by means of Sensor Data software (Wavefrontlabs), and (2) simultaneous video recording of the cows to allow accurate observation of the behaviours and their decomposition as sets of movements of the head or the jaw. The dataset was divided in two independent sets, one for calibration (the 3 first fields' data) and one for validation of the procedure (the 6 other data).

The data analysis included two steps and was performed using Matlab R2013a. The first task was to create an algorithm for the detection of the main behaviours: grazing vs. ruminating vs. other. This algorithm was based on criteria from the movements' decomposition on 3D-accelerometer and gyroscope signals. The results of the classification were compared with the observed behaviours on the validation dataset to calculate the detection accuracies. The second part of the work focused on rumination. The duration and the number of bites were counted on the video files. The interesting IMU signal, chosen according to the most discriminative movement for the rumination process, was analysed on time and frequency domain using fast Fourier transform (FFT) and its inverse. A first filtration process was performed by choosing the most recurrent frequency between 1Hz and 6Hz to eliminate noise waves from the raw data to bring out the actual signal from rumination. Two algorithms were developed to characterize the deglutition and the mastication (number and duration of deglutition, number and duration of bites). The deglutition was described as a pause between two bouts of mastication (mobile standard deviation of a 2s sample of the selected signal<0.03rad/s at deglutition) while the mastication was known by its duration ranging between 15s and 60s. For the mastication bouts, a second filtration between 1 and 2Hz corresponding to normal frequency of bites during rumination was done. The number of mastication is counted with the number of zero crossing waves on the filtered signal.

The foreseen results were compared with the observed data from the validation dataset.

Results and discussion

The detection accuracies for the grazing and rumination ranged between 90% and 100% on pasture and between 80% and 84% in the stable. The less-accurate detection in the stable is probably due to the different ration fed to the cows.

The rumination process was analysed using the rotation rate signal along the x-axis of the mobile device which is aligned with the cows nose to tail axis. This signal shows the particular jaw movement of the cattle during rumination best, showing a discriminant peak between 1 and 2Hz on the frequency domain (Figure 1). This behaviour is characterized by succession of 32 to 48s of mastication and 2 to 4s of pause for deglutition. As shown in Fig 2, correct measurements were high for both duration and number of mastications in which over estimation is not respectively greater than 7s and 7 bites for fields' data. For the number of mastications, a bite corresponds to four zero crossing waves. The average mastication rate equals to 1.06 ± 0.06 bites/s. For the stable sessions, the correct measurement yielded from the field data was much lower. The deglutition's standard deviation is lower than 0.03rad/s.
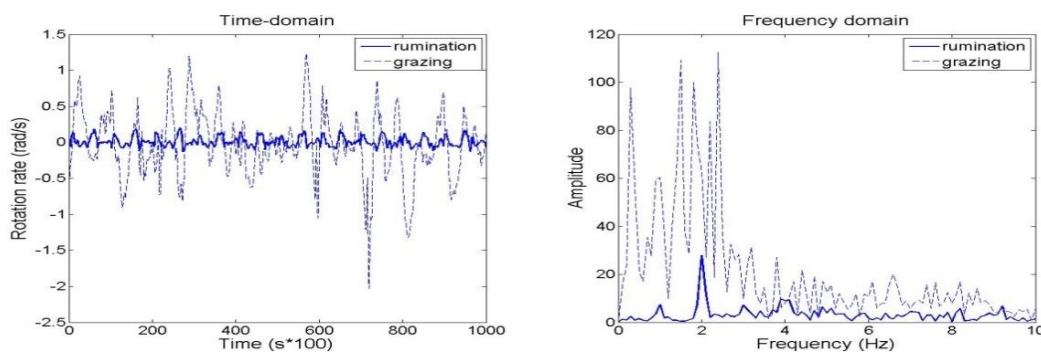

Figure 1. Time and frequency domain patterns for 10s mastication periods during rumination and grazing for field data along rotation rate on x-axis
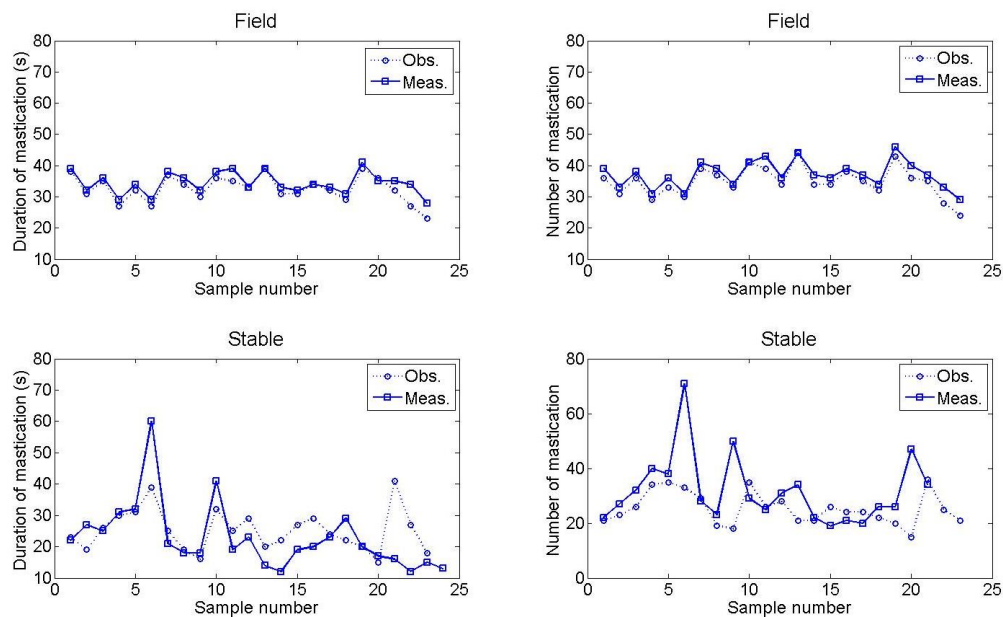
Figure 2. Comparison between the observed and the measured duration and number of mastication for field and stable validation data

The duration of mastication is also more scattered (between 15s and 40s). This situation is explained by the difference of the diets fed to the animals and requires further data processing to improve the algorithm.

Conclusion

The signals recorded from the IMU of iPhones and iPods offered an accurate detection of the behaviours of cattle. Deeper analysis about the rumination could measure its principal characteristics such as the duration and the number mastication but the approach requires further improvements to account for major changes in rumination patterns induced by differences between pasture and silage-based diets.

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Energy consumption and greenhouse gas emissions of DAIRYMAN farms in South-West Germany

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Abstract

The EU Interreg IVb NWE project DAIRYMAN was established in 2009. Its aim was to enhance the sustainability of dairying in North-West Europe by improving the competitiveness and ecological performance of dairy farming. Therefore, farm information - economic, ecological and social - was collected from a pilot farm network of 127 dairy farms in 10 regions of North-West Europe from 2009-2011 in order to evaluate and compare farm performances. Fouteen farms in South-West Germany (Baden-Wuerttemberg) participated in this network. In addition to the data that were collected for the whole network, the LAZBW Aulendorf determined energy consumption and greenhouse gas emissions for these German farms in 2010 and 2011. This was made with the AgriClimateChange Tool (ACCT), a newly developed EXCEL-based tool that enables the calculation of energy inputs and output, energy efficiency, nitrogen balances and greenhouse gas emissions. This tool is suitable for indicating the correlations of influencing factors, and to clearly visualize the farmers' efforts of adapting their farm management to climate-friendly systems.

Keywords: nitrogen balance, greenhouse gases, energy consumption, dairy farm system

Introduction

DAIRYMAN was an EU Interreg IVb NWE project which was established in 2009 with 14 partners under the lead of the University of Wageningen (Aarts, 2012). The project ended in August 2013. The main objective of DAIRYMAN was the investigation of dairy farming systems with regard to ecological, economic and social performances. This was realized by establishing a pilot farm network of 127 dairy farms. In Baden-Wuerttemberg, the German Dairyman partner, there were 14 farms which participated in the network. At the beginning of the project special development plans were worked out for each farm, so that the initial farm values could be compared with the values reached at the end of the project. With the AgriClimateChange Tool (ACCT), developed by 4 partners of the LIFE-funded project Agriclimate Change (Solagro, Bodensee Stiftung, Región de Murcia, Communità Montana and Fundacíon Global Nature (Solagro, 2013)), there is now a tool available which can be used throughout the European Union in order to show energy consumption and greenhouse gas emissions at a farm scale. In 2010 and 2011 such measurements with ACCT were made on the 14 German dairy farms. In particular, the influence of concentrates used and of the nitrogen inputs on the global energy efficiency needed to be investigated.

Material and methods

As basic information the ACCT (Solagro, 2013) uses the direct energy input (fuel, electric energy, water) and the indirect energy input (concentrates, fertilizer, machines, buildings, agricultural pesticides, seeds, animals and other synthetic materials). Products leaving the farm (like milk, meat and crops) are used for calculating the energy output. The 14 selected farms were not representative of the average dairy farms in Baden-Wuerttemberg, but they stand for typical farms in the region with successful milk production. Farms from four typical milk regions and different climate conditions were examined: Swabian Alb, Black Forest, Oberschwaben and Allgaeu. The selected farm types were divided into dairy farms with grass

feeding, dairy farms with maize feeding and dairy farms combined with biogas production. Details are reported in Table 1.

Table 1. Mean values, standard deviation, minimum and maximum values of selected farms for the attributes examined

Attributes	Unit	μ	±s	min	max
Agricultural area	ha	**123.9**	56.5	54.6	265
Agricultural area (AA)	ha	**92.6**	31.4	45.5	159.9
ECM	kg cow^{-1} y^{-1}	**8757**	932	6999	10150
Milk per farm area	kg ha^{-1}	**10106**	3656	5226	17346
Livestock units (LU)		**180.9**	67	79.1	280.6
Concentrate use in % of roughage	%	**25**	10	10	60
Fuel consumption	GJ ha^{-1}	**7.6**	2.3	4.2	11.2
Electricity per kg milk	kJ kg^{-1}	**10.3**	4.5	4.7	20,1
Feed purchase	GJ ha^{-1}	**8.5**	4.5	3.6	18.8
Fertilizer	GJ ha^{-1}	**5.5**	2.7	0	11
Input total	GJ ha^{-1}	**37.1**	10.3	20.3	55.4
Milk	GJ ha^{-1}	**26.8**	11.8	11.3	47.5
Meat	GJ ha^{-1}	**2.8**	1.3	1	5.7
Cultures	GJ ha^{-1}	**35.2**	45.1	0	133.7
Output total		**64.9**	38.4	22.7	147.5
Global energy efficiency (GEE)		**1.71**	08	1	3.4
t CO$_2$	ha AA^{-1} y^{-1}	**9.8**	3.3	5.8	15.7
t CO$_2$	LU^{-1} y^{-1}	**6.3**	1.2	4.5	9.3
Energy efficiency dairy branch		**1.07**	0.1	0.8	1.2
N balance	kg ha^{-1}	**80**	44.9	-5	170.5

Results and discussion

The energy consumption for fuel, fertilizer and feed purchase varied widely between the farms. Focusing on the global energy efficiency (GEE), which includes the branches milk, crop and biogas production, the differences between farms were remarkable. Dairy farms with biogas production had a 2 - 3 times higher GEE than dairy farms without biogas production. Only farms with biogas reached GEE values higher than 2.0. The energy efficiency for the dairy branch ranged between 0.8 and 1.2. It is remarkable that there is no close relation between GEE and nitrogen fertilization (Figure 1) and between GEE and the use of concentrates (Figure 2). Fertilizer used in energy input was only 15%, and it was obvious that the energy consumption from feed, electricity and fuel had more influence on the total energy input. Nevertheless, Figure 2 shows that increasing amounts of concentrates reduced the GEE.

Regarding only the dairy branch of the farms, it seems that the amounts of concentrates are positively correlated to the energy efficiency (Figure 3). This may be due to a more efficient milk production with higher amount of concentrates. Energy efficiency is increasing until a milk performance of 4000 kg milk from roughage. More milk from roughage seems to have no further benefits in terms of energy efficiency.
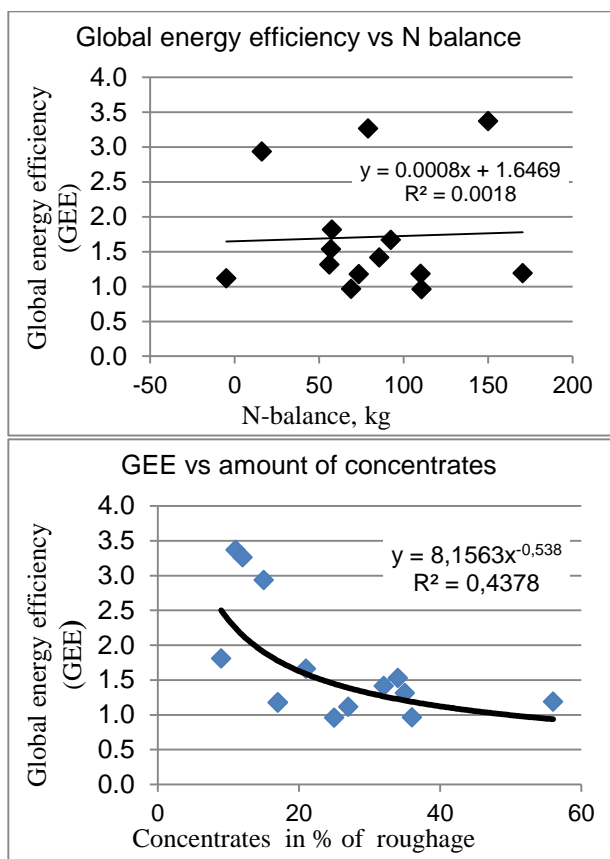
Figures 1 and 2: Relation between global energy efficiency and N balance respectively portion of concentrates in roughage
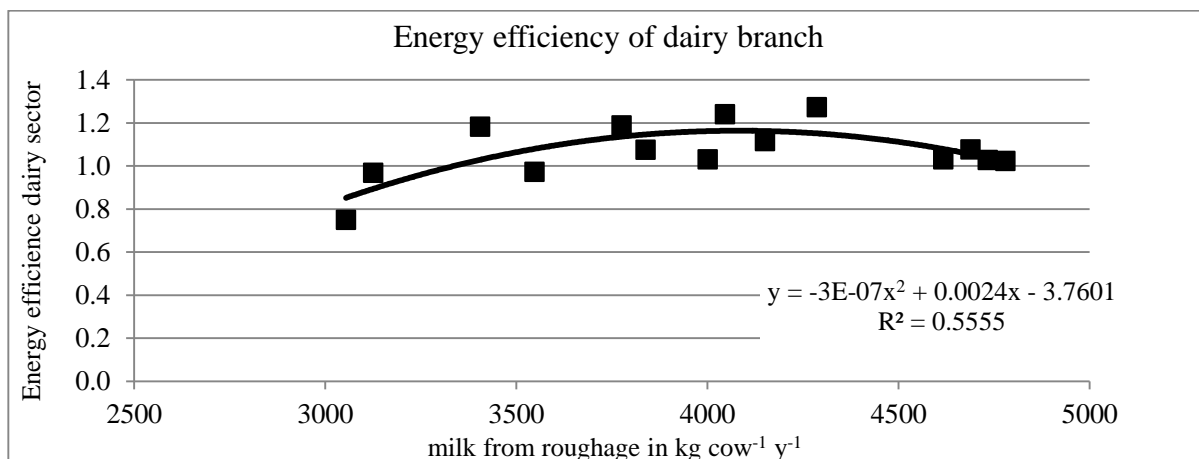

Figure 3: Energy efficiency of dairy sector related to milk performance from roughage

Conclusions

The dairy branches of the investigated farms differ in energy input, output and efficiency. This gives potential for improvements in optimizing the management of the farms on an individual basis, and it shows that intensive as well as extensive farms can be managed efficiently. The use of ACCT might be a helpful tool for farmers and extension services by showing strengths and weaknesses in terms of energy consumption of the individual farm.

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The DAIRYMAN-Sustainability-Index (DSI) as a tool for comparing dairy farms

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Abstract

Sustainability of dairy farms is determined by a multiplicity of single indicators. A combination of these single factors established useful sustainability indicator systems, but Von Wiren-Lehr (2001) report the truth of sustainability is not possible even if complex models or time consuming measurements are used. Therefore the team of the Interreg IVb NWE project Dairyman chose a pragmatic way to install an indicator system for comparisons of so-called sustainability of dairy farm systems. The number of indicators for the calculation of the DSI was substantially reduced on 17 indicators. The DSI is not yet a fixed system, but it can be a proposal for an evaluation of sustainability.

Keywords: sustainability, dairy farming, nutrient balances, farm economics

Introduction

Sustainability indicator systems have been established that combine a multiplicity of single indicators (Girardin, 2001; Belanger *et al.*, 2012), for example KUL (Breitschuh and Eckert, 2006), RISE (HAFL, 2012) or MOTIFS (Meul *et al.*, 2008). But how can the sustainable development of an individual farm be visualized and assessed? And why is it useful? A single characterization of 'sustainability' indicators does not give a good view of the whole-farm situation. More attractive, and of greater information value for farmers and advisors, is the combination of single factors in a so-called integrative view (Von Wiren-Lehr, 2001). An objective of the Interreg IVB NWE project DAIRYMAN (Aarts, 2012) was to create a management tool which is suitable for evaluating the development of sustainability of dairy farms as a combination of single indicators. This should visualize individual farm developments with the possibility to show differences in dairy systems in time and region. The DSI represents a holistic assessment of the DAIRYMAN pilot farms. It can help to show strengths and weaknesses of dairy systems.

Materials and methods

A network of 127 pilot dairy farms was installed in the Dairyman project in order to measure and observe processes in practical dairy farming in various countries of North-West Europe. For this purpose a high number of farm data in 2009-11 were collected. In a first step the chosen indicators for the DSI were selected by the project partner in Germany, after intensive discussions and with the use of a questionnaire answered by pilot farmers, farm advisors and teachers of agricultural schools. In a second step, the factors were further selected and discussed between all partners of DAIRYMAN in meetings (Elsaesser *et al.*, 2013; Grignard *et al.*, 2013). Based on the 'three-pillar model' of sustainability, it was decided that ecological, economic and social aspects would be treated equally, so that in each pillar a maximum of 100 points could be reached. All chosen factors were clearly defined and it was decided that they should be already gathered within the pilot farm network of all regions in order to reach an acceptable cost-benefit ratio. Biodiversity and soil erosion-susceptibility, although they are important attributes, could not yet be taken into account.

Results

Even though the task of the DSI was to harmonize the scoring values between all partners in the DAIRYMAN project, this objective could not be realized until now because single indicators of the DAIRYMAN partners are differently evaluated according to their importance in different regions. At first, this problem seemed unsolvable, but there are solution approaches (Larochelle *et al*., 2007; Meul *et al*., 2008; Belanger *et al*., 2012). The individual scores as a result of the discussions are summarized in Table 1. It is the task of the DSI user to discuss and interpret the gained results in a second step.

Table 1. Indicator quantiles of DAIRYMAN farm data (2010)

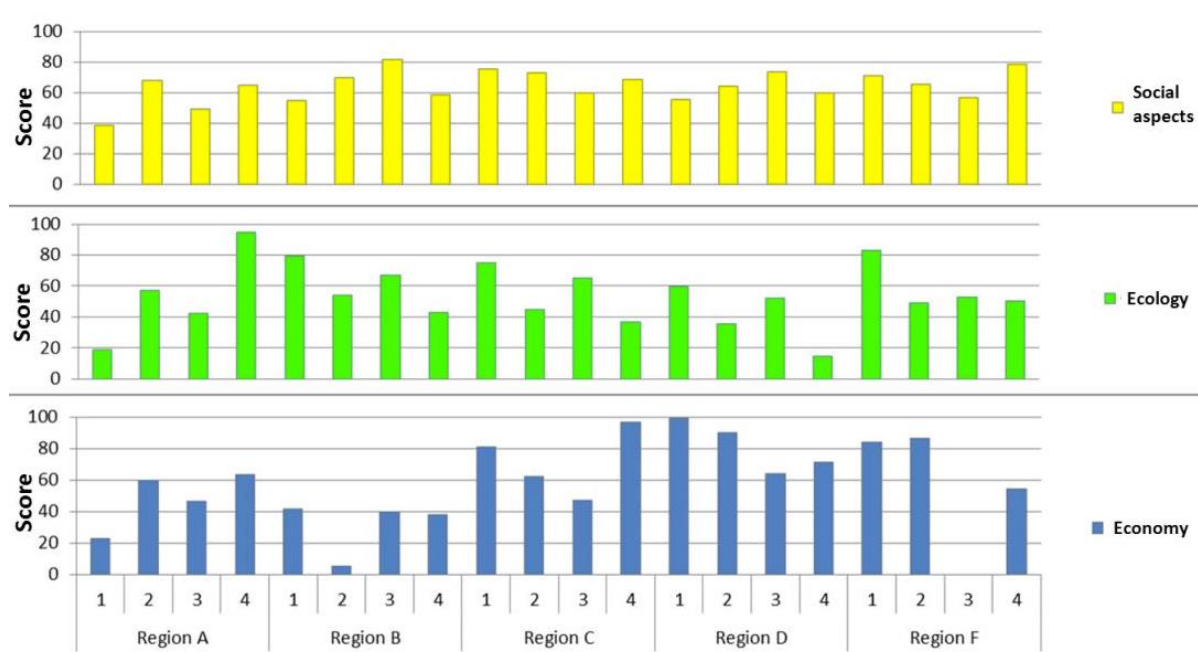| Indicator	Score	Minimum	10%quantile	90%quantile	Maximum
Economics (total)	= 100%				
Income per 100 kg/milk (€)	16	-7.6	2.6	23.7	34.9
Income per family worker (€)	34	-69427	13323	117467	202916
Farm income / family labour unit (€)	32	-69427	18081	109314	188543
Dependency on subsidies	10	-3.3	0.2	1.4	7.2
Exposure to price fluctuations	18	0.4	0.5	1.0	1.5
Ecology (total)	= 100%				
N Balance kg/ha	15	17.1	82.4	268.0	373.3
N Balance kg /1000 kg milk	11	3.9	9.1	34.3	60.9
N Efficiency %	13	11.8	19.4	47.5	64.4
P Balance kg/ha	11	-16.3	-4.6	17.9	43.9
P Balance kg/1000 kg milk	8	-4.6	-0.6	3.0	8.5
P Efficiency %	10	19. 5	35. 9	157.9	411.6
Agro-environmental Payments (€)	10	0.0	0.0	122.6	318.0
Greenhouse gas emissions	22	703.8	932.3	1427.7	1816.9
Social aspects (total)	= 100%				
Working conditions of farmers	42	These data were not worked out with quantiles. Scoring was made according to a multiple choice questionnaire (Elsaesser *et al*., 2013)			
Education	22				
Social role/image	20				
Continuity of farm	16				

Figure 1. Interregional comparisons of summarized scores of selected pilot farms

It was decided to take the quantile 10 and quantile 90 values of the complete 127-pilot-farm dataset as reference values for maximum and minimum scores. Pilot farms that are within the best 10% are awarded full marks for the particular indicator and farms within the worst 10% receive no points for the respective indicator. Points between these quantiles are calculated by linear regression. Multiplication of the measured values and the degree of target fulfillment give the score for each factor (Figure 1).

Conclusion

The DSI can be an integrative method in assessing developments of dairy farms. However, the aggregation of single factors has to be used carefully, because it is depending of a subjective scoring (Von Wirén-Lehr, 2001). It is no doubt, that the availability of a multi-annual data-set gives better results and a more solid analysis with lower sensitivity. The DSI offers a better insight on farm structures than comparisons of individual factors. Furthermore the DSI allows comparisons in dairy farm developments.

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Dairy system sustainability in relation to access to grazing: a case study

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Abstract

Extension of zero-grazing management scheme questions sustainability of dairy production. The aim of this work was to compare, during two seasons, the technico-economic and environmental performances of two experimental dairy herds with similar genetic potential. The first herd had full access to grazed grasslands during summer time while the second remained in the cowshed. Management scheme did not significantly affect dairy production, neither in quantity nor in quality. It only affected the distribution of the production across the year. From May till July, grazing cows produced, on average, significantly more milk than zero-grazing ones (22.7 vs. 19.7 kg d^{-1} cow^{-1}; $P = 0.02$) while the opposite was true from November till January (17.8 vs. 20.4 kg d^{-1} cow^{-1}; $P = 0.03$). From the economic point of view these results underlined a huge increase in total production costs (feeding, bedding and reproduction costs), with an average increase of 30%, across both years, when shifting from a grazing to a zero-grazing management scheme (22.8 ± 4.0 and 29.7 ± 4.5 € per 100 kg of standardized milk). From the environmental point of view, the zero-grazing system had better nitrogen balances with, on average and across both years, 112 kg N compared with 131 kg N ha^{-1}. In term of climate footprint, the grazing system led to lower emissions of greenhouse gases than the zero-grazing system due to the highest dependency of the zero-grazing system on fertilizer and feedstuff inputs. This result is of value whatever the functional unit mobilized, with 8550 ± 595 and 10700 ± 208 kgCO$_{2eq}$ ha^{-1} and 1140 ± 144 and 1350 ± 76 kgCO$_{2eq}$ ton of milk^{-1}, for grazing and zero-grazing systems respectively. Based on these results, grazing-based systems appeared to be more sustainable than zero-grazing systems.

Keywords: zero-grazing, GHG, N balance, dairy performance, DAIRYMAN

Introduction

As grasslands cover 50% of agricultural area in Wallonia, they play a major role in dairy production. Nevertheless, as in many European countries, zero-grazing systems are becoming more numerous. From a farmer's point of view, such management scheme allow improved control of diets, optimize grassland utilization, and achieve a higher milk production, higher labour efficiency, and lower nutrient losses (Meul *et al.*, 2012). Grassland scarcity and/or accessibility, proportionally to herd size, or the use of a milking robot can also support the adoption of a zero-grazing management scheme (Arsenault *et al.*, 2009). Nevertheless, such a scheme can increase dependency on external feedstuffs and feeding costs.

The aim of this work was to compare, during two years, the technico-economic and environmental performances of two experimental dairy herds with similar genetic potential under contrasting management schemes: the first herd had full access to grazed grasslands during summer time while the second remained in the cowshed.

Materials and methods

Cows were allocated to the two herds in order to obtain balanced groups with similar parity distribution. Calving occurred all year long. Breeding phase, till first calving, was common to both herds. Heifers grazed as soon as the weather was favourable, as did dry cows of both herds. After calving, the heifers were introduced into their mother herd.

Grazing management was based on a rotational scheme with residence time of 3.6 days, on average, and 37.7 days between two grazing periods. On average, 60 kg of mineral N were applied per ha and per year, to complement the slurry spreading. This fertilization scheme allows the development of a significant proportion of clover in the sward. System characteristics are presented in Table 1.

Table 1. General characteristics of the two dairy systems compared

Parameters	Unit	Zero grazing		Grazing maximization	
		2010-11	2011-12	2010-11	2011-12
Average herd size	cows	25.5	27.5	22.5	22.5
Cow's average weight	kg	-	642.6	-	641.8
Agricultural area for the herd	ha	21.1	22.7	18.4	19.4
Grassland area	ha	12.9	10.5	13.1	15.5
Grazed grasslands (GG)	ha	4.8	4.1	8.0	8.7
Maize (other cereals)	ha	7.7 (0.5)	7.9 (4.3)	3.3 (2.0)	2.9 (1.1.)
Cereals	ha	0.5	4.3	2.0	1.1
Stocking rate in GG	LU ha^{-1}	3.8	3.5	3.7	3.6
Maize silage in the diet[a]	%	35	42	17	17
Concentrate in the diet[a]	%	25	31	16	19
Grass in the diet[a]	%	34	21	63	61

[a]Annual average, on a DM basis. Concentrates are allocated on an individual basis, linked to the production potential of the cow at the rate of 1 kg of concentrate per 2.5 kg of milk over the 22 kg of daily production. Intake level, under grazing, was estimated through faeces analysis by NIRS (Decruyenaere *et al*. 2012).

Animal performances were reflected by their annual production and the monthly profile of production of each herd.

A simplified economic balance was calculated for both herds, excluding either subsidies and the following costs: water, electricity, taxes, insurance, administrative costs, rental costs, interest and depreciation.

Finally, environmental pressure was quantified through N, P and K balances and quantification of emissions of Greenhouse Gases based on IPCC methodology (Tier 2) as defined by the DAIRYMAN project (Aarts *et al.,* 2013).

While animal performance characteristics led to statistical comparisons, the economic and environmental parameters, applied at the herd scale, allowed only descriptive approaches.

Results and discussion

Even though zero-grazing cows had, on average, a higher level of production (7868 (+ 8 %) vs. 7286 kg per cow) than grazing cows, the difference was not significant ($P = 0.29$). In the same way, the management scheme did not affect the monthly milk production per cow ($P = 0.41$) which turned out to be affected primarily by the season ($P = 0.04$) and this in a different way in each system (P season×system $= 0.01$) independently of the year ($P = 0.60$). Indeed, from May till July, grazing cows produced, on average, significantly more milk than zero-grazing cows (22.7 vs. 19.7 kg d^{-1} cow^{-1}; $P = 0.02$) whereas the opposite was true from November till January

(17.8 vs. 20.4 kg d^{-1} cow^{-1}; $P = 0.03$). The protein (3.4 g kg^{-1} of milk) and the fat (4.1 g kg^{-1} of milk) contents were not affected either by the management system ($P= 0.14$ and $P = 0.28$, respectively) or by the interaction between the management system and the season ($P = 0.16$ and $P = 0.97$, respectively).

These results support earlier findings on the stability of production in zero-grazing systems, as grazing animals have to face resource variability, both in quantity and quality, across the season. Nevertheless, for animals with similar genetic potential, no significant production difference was highlighted on a yearly basis.

From the economic point of view, the results underlined a huge increase in total production costs (feeding, bedding and reproduction costs), with an average increase of 30% across both years, when shifting from a grazing system to a zero-grazing management scheme (22.8 ± 4.0 and 29.7 ± 4.5 € per 100 kg of standardized milk).

Due to its lower autonomy, the zero-grazing system also had higher N (+38 %), P (+ 33 %) and K (+ 35 %) inputs, which were compensated by higher exports, expressed per ha. This led to better mineral balances in zero-grazing systems with, on average across both years, 112 against 131 kg N ha^{-1}; 6 against 19 kg P ha^{-1} and 98 against 104 kg K ha^{-1}. Meul *et al.* (2012) underlined lower level of N surpluses in grazing than in zero-grazing commercial farms in Flanders, but our average values are below their lowest benchmark value of 136 kg N ha^{-1}.

In terms of climate footprint, the grazing system led to lower emissions of greenhouse gases than the zero-grazing system due to the highest dependency of the zero-grazing system on inputs of fertilizer and feedstuffs. This result is of value whatever the functional unit mobilized, with 8550 ± 595 and 10700 ± 208 kgCO$_{2eq}$ ha^{-1} and 1140 ± 144 and 1350 ± 76 kgCO$_{2eq}$ ton of milk^{-1}, for grazing and zero-grazing systems respectively.

Conclusions

Based on these results, grazing-based systems appeared to be more sustainable than zero-grazing systems and would allow for an improved buffering capacity against price volatility. Nevertheless, they require the availability of easily accessible grassland paddocks to support the herd size increase, as the production per hectare is higher in zero-grazing than in grazing systems (with 11600 vs. 9900 kg of milk ha^{-1}). A good knowledge of herbage management is also necessary, in order to optimize grassland productivity.

Acknowledgements

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Beef productivity on the North Wyke Farm Platform in two baseline years

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Abstract

The North Wyke Farm Platform (NWPF) has been implemented to deepen the understanding of sustainable grassland management systems, including the impacts on livestock productivity. Two baseline years of data were collected (2011 and 2012) to establish the level of productivity of the existing permanent pasture on three hydrologically isolated farmlets. Cattle were put on the platform at weaning and remained until finishing. They were housed in winter and grazed on a continuous-stocking basis during the grazing period. Records of inputs, such as nitrogen fertilizer and farmyard manure, were taken along with regular monitoring of liveweight gain. Overall average daily gain (ADG) from weaning to selection for sale was 0.7 kg/day (± 0.01 SEM) in 2011 and 2012, and there were no significant differences between farmlets in either year. Silage DM yield was lower in 2012 due to heavy rainfall and low levels of solar radiation. The equal performance of cattle on the three farmlets, under existing permanent pasture, will allow future differences, when the new management systems are operational, to be accounted for by the effects of the new systems.

Keywords: North Wyke farm platform, beef production, sustainable grassland, permanent pasture

Introduction

The Foresight Report (2011) highlighted the need for sustainable intensification of livestock production. However, there is a lack of understanding of the impact of grassland systems in terms of pollution and potential benefits, e.g. carbon sequestration. The North Wyke Farm Platform (NWFP) is a national capability developed to improve our understanding of intensive, pastoral ruminant livestock production systems with respect to their impact on the environment and value in contributing to global food demand and providing healthy nutritious human food (Murray *et al.*, 2013). The NWFP provides a farm-scale facility with extensive instrumentation to collect core parameter data to assess the impacts and benefits of three different pastoral systems, each providing alternative approaches to potentially productive grassland farming. The first is a continuation of the existing permanent pasture improved with N-fertilizer application, the second is the introduction of N-fixing legumes and subsequent greatly reduced application of artificial N-fertilizer; the third is the introduction of innovative new species such as deep rooting *Festulolium* through planned reseeding. The effects of these systems on water, air and soil will be monitored. The productivity of the cattle and sheep on the platform is a vital consideration in terms of the inputs and outputs of the grassland system. Before introduction of the new management systems from spring 2013, baseline data were collected for two years to establish the level of beef production on the existing pasture on the NWFP, which will be critical to establish causative effects of the new imposed systems in future years.

Materials and methods

The farm platform comprises three farmlets, each approximately 21 ha. The platform has been instrumented to collect various core parameter data including analysis of water samples (collected via the French-drainage system installed around the perimeter of each field), soil conditions and atmospheric measurements. Detailed records of all inputs and outputs to the farm platform were maintained. N fertilizer was applied according to DEFRA RB209 (Defra, 2013) and NVZ guidelines, when conditions were suitable. Farmyard manure (FYM) was

applied following cutting. Cattle were grazed on a continuous stocking basis, maintaining sward surface height at approx. 6-9 cm (Hodgson *et al*., 1986). Animal performance was measured by regular weighing. Two years (2011 and 2012) of baseline data for beef performance were analysed to establish the level of production under the current management of permanent pasture. The existing pasture has been established across the platform for at least 10 years. Swards were surveyed for plant species abundance in summer 2013 and found to contain percentage covers (Rodwell, 1992) of: 64 *Lolium perenne*, 38 *Agrostis stolonifera*, 2 *Holcus lanatus* and 1 *Alopecurus geniculatus* as the main constituents.

Results

The farm platform carried 75 (28 heifers and 47 steers) and 81 cattle (38 heifers and 43 steers), 25 and 27 per farmlet in 2011 and 2012, respectively. There were also 15 younger calves in 2012; however, final data for these were not available at the time of writing. The cattle were weaned, housed and weighed in October. The average wean weight and age were 300 kg (± 5.44 SEM) and 180 d (± 4.00 SEM) in 2011 and 319 kg (± 5.31 SEM) and 212 d (± 7.8 SEM) in 2012. There were various breeds including: Aberdeen Angus, Simmental, Limousin, Hereford and Charollais crosses. The cattle were turned out on 13 April in both 2011 and 2012 with mean weight 391 kg (± 7.06 SEM) and 395 kg (± 5.43 SEM), respectively. Stocking rates on the whole NWFP were 1.3 and 1.7 cattle /ha but 5.8 and 5.9 cattle/ha on grazed areas, at time of grazing for 2011 and 2012, respectively. In 2011 the grazing period was 204 days; 30 cattle that were not finished at grass were housed on 3 November 2011 (mean weight 558 kg ±8.55 SEM).

Table 1: Mean liveweight gain of beef cattle from weaning to finishing on the NWFP in two baseline years (ADG = average daily gain; FP = farm platform).

2011 Farmlet	1	2	3	*P* value	FP	Average
ADG (kg) (1st winter, housed)	0.54	0.50	0.54	0.658	0.53	
SEM	0.039	0.033	0.032			0.017
ADG (kg) (grazing period)	1.05	0.99	1.01	0.459	1.02	
SEM	0.039	0.042	0.018			0.022
ADG (kg)(2nd winter, housed)	0.70	0.81	0.78	0.760	0.76	
SEM	0.121	0.12	0.056			0.063
Overall ADG (kg)	0.72	0.70	0.73	0.394	0.72	
SEM	0.017	0.017	0.018			0.010
2012 Farmlet	1	2	3	*P* value	FP	Average
ADG (kg) (1st winter, housed)	0.46	0.39	0.46	0.283	0.44	
SEM	0.041	0.024	0.033			0.019
ADG (kg) (grazing period)	0.93	0.83	0.91	0.155	0.89	
SEM	0.032	0.027	0.032			0.018
ADG (kg) (2nd winter, housed)	1.35	1.33	1.21	0.712	1.30	
SEM	0.162	0.106	0.061			0.068
Overall ADG (kg)	0.74	0.68	0.74	0.089	0.72	
SEM	0.019	0.02	0.023			0.013

Farmlets: 1 = Planned reseeding, 2 = Improved permanent pasture, 3 = Increased legume utilization.

The 2012 grazing season was 166 days; 48 cattle that were not finished at grass were housed on 3 October 2012 (average weight of 537 kg ± 7.89 SEM). Mean age at selection for sale was 591 d (± 9.0 SEM) and 617 d (± 10.39 SEM); mean number of days between weaning and selection was 401 d (± 6.6 SEM) and 379 d (± 6.15 SEM) for 2011 and 2012, respectively. There were no significant differences between farmlets for live weights, age or days to selection in either year. ADG for both years is shown in Table 1, with no significant differences between farmlets observed. Mean age at selection was significantly higher in 2012 than 2011 (*P*=0.016), number of days from weaning to selection approached significance and was higher in 2011 (*P*=0.055).

The total annual N fertilizer applied was 201 and 83 kg/fenced ha, with 11 and 8 kg/fenced ha of FYM N applied following cutting, for 2011 and 2012, respectively. In 2011 there was 63 ha cut for silage (1st & 2nd cut); average yield was 5.9 t DM/ha, a total of 369 t DM harvested with mean DM of 32.6%. In 2012 there were 39 ha cut for silage (1st & 2nd cut); average yield was 4.1 t DM/ha, a total of 157 t DM harvested with mean DM of 23.6 %.

Discussion

After April 2013 the three farmlets began to have different grassland management approaches as the new treatments are progressively rolled out. These base-year data demonstrate that there is no significant difference in performance of stock on each area under established permanent pasture, suggesting nutritional quality and herbage provision is equal across the platform and providing evidence that any future differences in performance observed will be the result of the different management systems. According to best-practice guidelines (EBLEX, 2008, 2005; http://www.eblex.org.uk/publications/), the target ADG at grass should be at least 0.8 kg/d. This was achieved on the farm platform in both years showing that the level of production is good in comparison with industry targets. The significant difference seen between years for age at selection for sale can be accounted for by the cattle being 1 month older at weaning in 2012 than 2011.

Silage DM yield per hectare was lower in 2012, which was a result of reduced DM content of silage due to the significantly higher rainfall and low levels of sunshine for that year. N fertilizer application was less in 2012 in response to initial good spring growth and then poor weather conditions. Mean daily rainfall between 1 April and 30 October was 1.97 mm ($\pm$ 0.250 SEM) and 3.42 mm ($\pm$ 0.426 SEM) in 2011 and 2012, respectively ($P>0.003$). Stocking rates were higher in 2012; therefore the area conserved was an emergent property, which was reduced because it was determined by requirements for grazing stock.

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Milk production of sheep fed on preserved forage in winter and grazing in spring

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Abstract

In Bulgaria there are 39 breeds of sheep reared primarily for milk. The purpose of this experiment was to test the effect of feeding rations based on maize silage, alfalfa hay and silage in the winter, and grazing on temporary and natural pasture in the spring, on the quantity and composition of sheep milk. Thirty sheep from the Pleven Blackface breed were used, divided into three groups. The experimental period of 84 days was divided into two sub-periods of 42 days. During the first sub-period the first group received maize silage, the second group alfalfa hay, and the third group alfalfa silage. In all rations, compound feed to cover the needs for 1.5 L milk was included. In the second sub-period, the first group grazed natural pasture, the third group grazed on temporary pasture of cocksfoot and sainfoin, and the second group remained on alfalfa hay. The sheep received 0.5 kg of grain maize during the grazing period. The highest daily milk yield was obtained by a feeding ration based on alfalfa hay, which was 14-26% higher compared to that from rations based on maize silage and alfalfa silage. The milk of sheep grazing on temporary pasture was 28.5% more than that of ewes grazing on natural pasture; this was probably due to the higher content of legumes in the temporary pasture. During the lactation period the quantity of milk decreased the least when grazing on temporary pasture.

Keywords: sheep, feeding, milk, composition, pastures

Introduction

The number of sheep in Bulgaria until 1990 varied between 7 and 11 million, but in the period of democratic changes their number have decreased over five times. The development of sheep breeding is due to the presence of huge natural resources of meadows and pastures, which represent 28% of the country's agricultural area. In Bulgaria, 39 breeds of sheep are reared and half of them are local breeds for milk. Sheep milk is processed primarily into cheese, which sells well on the local market and abroad. The production of more sheep milk and the economic prosperity of the farm are related not only to the improvement of the genetic potential of the sheep, but also to the improvement of the nutrition and the farming systems. The purpose of the experiment was to test the effect of feeding rations based on maize silage, alfalfa hay and alfalfa silage in the winter, and grazing on temporary and natural pasture in the spring, on the quantity and composition of milk from sheep.

Materials and methods

The experiment was carried out in 2013 at the Institute of Forage Crops, Pleven. For this purpose, 30 sheep from the Pleven Blackface breed were used, divided into 3 groups. The lambs were weaned 25 days after birth, and the sheep entered the experiment 35 days after lambing. The experimental period lasted 84 days and was divided into two equal sub-periods of 42 days (6 weeks). In the first sub-period, the first group received *ad libitum* maize silage, the second group received alfalfa hay, and the third group received alfalfa silage. The sheep in the three groups received compound feed of sunflower meal, rapeseed meal, maize grain, triticale and vitamin-mineral supplement to cover the needs of daily milk yield of 1.5 L, according to Todorov and Dardjonov (1995). In the second sub-period, the first group was moved from maize silage to grazing on natural pasture, and the third group went from alfalfa silage to grazing on temporary pasture. During grazing, both groups received 0.5 kg of grain maize per

sheep. The temporary pasture was of cocksfoot (*Dactylis glomerata*) and sainfoin (*Onobrychis viciifolia*). During the experimental period, the daily milk yield from each group was controlled and on two consecutive days of the week the individual milk yield per ewe was also controlled. The milk composition was determined by Milko Scan, model 133. Samples were taken of the natural and temporary pastures to determine the botanical and chemical composition. The dry matter (DM), crude protein (CP), crude fibre (CF), fat, ash and nitrogen-free extract substances (NFE) in the forages were determined.

Results and discussion

In the first sub-period of the experiment, the highest average daily milk yield was obtained from the second group of sheep, fed on a ration based on alfalfa hay (1.322 L; *P*> 0.05) (Table 3). When fed on ration based on alfalfa hay, the sheep gave 14-26% more milk compared to the group fed rations based on maize silage and alfalfa silage (*P*> 0.05). Between the first and the third groups, no significant differences in quantity of the milk were observed. In the second (grazing) sub-period the highest average daily milk yield (1.001 L) was observed in the group grazing on temporary pasture (*P*> 0.05). The sheep grazing on natural pasture and those which continued to feed on alfalfa hay, had lower milk yields than the group grazing on temporary pasture. The total milk in the second sub-period for the group grazing on temporary pasture was 28.5% and 25.8% higher than that of the sheep from the other two groups, i.e., grazing on natural pasture and alfalfa hay (*P*> 0.05) respectively. During the second sub-period compared with the first sub-period, the amount of milk in the third group (grazing on temporary pasture) decreased by 4.5% only, while the first group grazing on natural pasture and the second group fed on alfalfa hay the drop was 31.4% and 40%, respectively.

The first sub-period of feeding and milking of the sheep coincides with the period after lambing, when the sheep give the most milk. Similar average daily milk yields in ewes of the same breed and in the same period of lactation were obtained by Kirilov *et al.* (1998) and after feeding on silage of alfalfa and peas (Kirilov and Simeonov, 2012). After the first two months of lambing the sheep milk yield decreases. This decrease is not significant in the group grazing on temporary pasture, which has high proportion of the legume component and a high content of crude protein (Tables 1 and 2).

Table 1. Chemical composition of rations and forages, g kg⁻¹ DM

Forages	DM	CP	CF	Fat	Ash	NFE
Ration 1 gr.		148.98	178.37	21.33	53.02	598.30
Ration 2 gr.		140.85	216.10	18.50	57.10	567.45
Ration 3 gr.		146.82	218.53	24.55	63.47	546.63
Temporary pasture						
1-3 week	177.20±0.32	256.70±1.67	155.16±3.38	48.00±0.153	106.00±1.23	434.14±3.11
3-6 week	210.20±2.07	179.50±1.43	244.70±0.53	35.10±0.22	113.10±0.46	427.60±1.47
Natural pasture						
1-3 week	187.9±0.99	202.20±1.04	223.00±0.18	42.90±0.18	124.40±0.65	407.50±0.13
3-6 week	266.00±5.64	176.50±0.22	308.80±0.15	31.10±0.26	91.80±1.96	391.80±0.68

Table 2. Yield and botanical composition (grass:legume:other) temporary (TP) and natural (NP) pastures

	1 week	2 week	3 week	4 week	5 week	6 week
TP, kg ha⁻¹	1497.3±108.4	1637.2±77.7	3594.1±68.7	6480.3±69.5	8503.5±157.6	10012.3±187.8
gr:leg:other	32:68:0	41:58:1	42:52:6	45:50:5	50:46:4	54:40:6
NP, kg ha⁻¹	967.6±148.9	1000.1±107.1	1177.0±134.0	1315.6±81.0	1923.0±109.4	2125.6±170.2
gr:leg:other	95:0:5	92:4:4	87:7:6	83:10:7	80:11:9	79:12:9

As regards the composition of milk, no significant differences were detected between the indicators of fat and proteins between the groups during the first sub-period. In the second sub-period, an increased concentration of fat and protein in the milk is observed, compared to the first. This is likely due to the lower daily milk yield (Fuertes *et al.,* 1998).

The content of CP in temporary pasture at the beginning of grazing, 1-3 weeks, was high at an average of 256.7 g kg⁻¹ DM, and decreased over the next three weeks to 179.5 g kg⁻¹ DM, and the contents of CF increased from 155.2 g kg⁻¹ DM and 244.7 g kg⁻¹ DM (Table 1) ($P> 0.05$). During the same period, the contents of the CP in the natural pasture during the first 3 weeks, were lower than in the temporary pasture ($P <0.05$) and the content of CF was higher ($P <0.05$). A similar trend for low CP and high on CF in natural pasture was observed in weeks 3-6 compared to weeks 1-3 of the grazing period. Higher content of CP and low content of CF at the beginning of the grazing period (weeks 1-3) are regular; subsequently, with the advancing of the vegetation period, there is a decrease in the CP and increase of the CF. The high levels of CP in the temporary pasture compared to natural pasture is probably due to the higher proportion of the legume component, 40-68% compared with 0-12% in the natural pasture (Table 2). This is probably the reason for higher milk yield in the group grazing on temporary pasture, compared to the group grazing on natural pasture.

Table 3. Milk production and composition. Different superscript letters in columns indicate differences are significant at *P> 0.05*

Group	Daily milk yield, l	Total milk per sheep, l	Fat, %	Protein, %	Lactose, %	Total Solids, %
First sub-period						
1 maize silage	1.155[c]±0.048	48.49[b]±0.22	5.15[a]±0.63	5.63[ab]±0.39	5.31[c]±0.25	16.50[a]±1.08
2 alfalfa hay	1.322[d]±0.040	55.55[c]±0.34	5.31[a]±0.98	5.83[b]±0.38	5.50[d]±0.26	17.12[a]±1.17
3 alfalfa silage	1.049[bc]±0.027	44.06[b]±0.19	5.22[a]±0.79	5.63[ab]±0.50	5.50[d]±0.17	16.83[a]±1.20
Second sub-period						
1 nat. pasture	0.779[a]±0.058	32.71[a]±0.43	8.45[c]±0.91	5.42[a]±0.32	4.88[a]±0.14	19.35[d]±0.96
2 alfalfa hay	0.795[a]±0.068	33.41[a]±0.48	6.77[b]±0.60	6,15[c]±0.34	5.11[b]±0.41	18.63[c]±0.60
3 temp. pasture	1.001[b]±0.042	42.05[b]±0.29	6.27[b]±0.57	5.73[b]±0.40	5.31[c]±0.19	17.92[b]±0.85

Conclusions

The sheep fed a ration based on alfalfa hay gave 14-26% more milk, compared to the milk yield of sheep fed on maize silage and alfalfa silage. Sheep grazing on temporary pasture with higher content of legumes and crude protein gave 28.5% more milk compared to those grazing on

natural pasture. During the course of the lactation period, the quantity of milk decreased the least when sheep were grazing on temporary pasture.

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Evaluation of a home-grown crimped lupin and barley concentrate feed for finishing lambs

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Abstract

To investigate whether a home-grown lupin-barley concentrate feed could support similar levels of lamb productivity and carcass quality to a commercial lamb-finishing diet, two iso-nitrogenous concentrates were offered to castrated male Texel-cross lambs. Diets comprised barley straw plus either: home-grown concentrate, viz. crimped narrow-leafed lupin (*Lupinus angustifolious* cv. Boruta), crimped barley (*Hordeum vulgare* cv. Propino) and sheep mineral (705, 270 and 25 g kg^{-1} dry matter respectively); or, a pelleted, commercial lamb finisher (Control). Straw and each treatment-diet were offered *ad libitum* over 56 d to 4 replicate pens of 5 lambs, totalling 20 lambs per treatment. Group intakes were recorded over days 0-28 and lambs were weighed and condition scored at 7-d intervals throughout the experiment. From day 29 onwards, individual lambs were selected out for slaughter and carcass characteristics were recorded. Results showed no differences in the productivity, concentrate conversion efficiency, days to finish or carcass characteristics of lambs offered either the crimped lupin-barley concentrate or control diet. Overall, the findings of this study show that home-grown crimped lupin-barley concentrate diets can be used to finish lambs without any detrimental effects on productivity or carcass characteristics when compared to a commercial lamb finishing diet.

Keywords: Lupin, *Lupinus angustifolius*, crimped grain, lamb, home-grown

Introduction

Livestock farmers worldwide are aiming to reduce reliance on imported and bought-in feedstuffs, which may be subject to world market price fluctuations and have a high environmental footprint. Oilseed rape and palm kernel cake and meal are typically used in commercial concentrate diets for sheep in the UK. Lupins (*Lupinus*; Leguminosae), although not traditionally grown as a field crop in the UK, yield grain with a potentially high nutritional value both in terms of protein content (compared with peas or beans) and oil content, which give them high energy value. Previous work has shown that both narrow-leaf and yellow lupins can be used within a pelleted concentrate for finishing lambs instead of either soya or compared to a control lamb finisher diet, without any adverse effects on lamb productivity or carcass killing-out percentages (Fychan *et al.*, 2008). However, this approach requires the use of grain-drying facilities which are not often available on livestock-based farms. An alternative approach to storing lupins is the use of crimping technology to preserve the grains and which overcomes the need for grain drying facilities and also allows for the crop to be harvested at an earlier date and stage of maturity. In crimping, grain is harvested moist (approx. 60 per cent dry matter content), passed through a crimper (to flatten and open the grains) and preserved using an additive before being ensiled. Here, we present the findings of a study to investigate the effects of incorporating crimped narrow-leafed lupins into the concentrate diets of finishing lambs, on lamb productivity and carcass characteristics when compared to a commercial lamb finisher diet.

Material and methods

Forty castrated male Texel-cross lambs were sourced from the same late-lambing flock for the experiment. The experiment involved a 14-d standardization period followed by a 14-d

adaptation period and 56-d measurement period (initial live weight 32 ± 2.8 kg). The two dietary treatments were a home-grown mixed ration comprising crimped narrow-leafed lupin, crimped barley and sheep minerals (705, 270 and 25 g kg^{-1} dry matter (DM) respectively) and control diet of commercial lamb finisher pellets. The home-grown ration was formulated to be iso-nitrogenous with the control concentrate. The lupin (*Lupinus angustifolious* cv. Boruta) and barley (*Hordeum vulgare* cv. Propino) for the experiment were established at the same site at Aberystwyth University on 16 April 2013 and their grain was harvested at a target DM content of 700 and 600 g kg^{-1}, respectively. Moist barley grain was harvested on 8 August and moist lupin grain was harvested on 27 August 2013. Immediately after harvest, the grain was rolled through a Murska 350 S2 crimper (Aimo Kortteen Konepaja Oy, Ylivieska, Finland), and Crimpstore 2000S preservative (Kemira Oyj, Helsinki, Finland) was applied to the barley and lupins at the rate of 4 and 6 l t^{-1}, respectively. Quantities of each feed were stored in sealed polythene bags within 500 kg tote bags to provide an amount that was sufficient for 7 days of feeding.

During the standardization period, the lambs grazed a typical ryegrass-clover sward as a single group. At the start of the adaption period, lambs were allocated to treatment, and pen within treatment, on the basis of live weight and body condition score. The eight groups of five lambs were then housed in pens with sawdust bedding and dietary treatments and barley straw were introduced gradually. Fresh water was available at all times. Throughout the measurement period, barley straw and the treatment concentrate diets were offered *ad libitum* with a refusal margin of 0.10 to 0.15 d^{-1}. Pen intakes were monitored over days 0-28 and lambs were weighed and condition scored at 7-d intervals throughout the experiment. Live-weight gain between days 0 and 28 was recorded using live weight recorded on two successive days at the start and end of this period. From day 29 onwards, lambs were selected-out for slaughter as they reached a target fat class of 3L and their live weight and carcass characteristics were recorded. Prior to slaughter, back fat and muscle depths were measured by ultrasound at the third lumbar vertebra. Killing-out ratio was calculated as the ratio of cold carcass and live weights. All data were analysed by ANOVA except days-to-finish-date, which were analysed by sign test, using Genstat® Version 14.2 (Payne *et al*., 2011). Pens were treated as the experimental unit in all analyses.

Results and discussion

The chemical analysis of the concentrate feeds are presented in Table 1. The data confirm visual observations that both crimped diets were well preserved, with similar dry matter (DM), nitrogen and ME values to previously reported data for lupins by Fraser *et al.* (2005). The results of the effects of each treatment diet on lamb productivity and carcass characteristics are presented in Table 2. The median time to finish was 29 d for the control diet and 32.5 d for the home-grown diet ($P > 0.05$). Results showed no differences in the productivity, concentrate conversion efficiency, days to finish or carcass quality characteristics of lambs offered either the crimped lupin-barley concentrate or the control diet.

Table 1. Chemical analysis on concentrate feeds (g kg^{-1} DM unless otherwise stated)

	Crimped Barley		Crimped Lupin		Control	
	Mean	s.d.	Mean	s.d.	Mean	s.d.
Dry matter (g kg^{-1})	578	9.0	795	0.8	901	16.3
Ash	19.9	0.10	35.2	0.60	87.9	9.72
Nitrogen	18.4	0.20	52.2	1.85	27.0	0.50
Oil	16.7	1.05	57.3	1.33	40.8	1.39
Crude fibre	72	8.4	215	10.5	158	6.0
NGCD	879	13.8	950	1.5	765	4.3
ME (MJ kg^{-1} DM)	12.7	0.20	14.7	0.05	11.7	0.09

NCGD = Neutral cellulase gammanase digestibility; ME = Metabolizable energy

Table 2. Lamb productivity and carcass characteristics in relation to dietary treatments

	Home-grown concentrate	Control	s.e.d.[$]	*Prob*
Concentrate intake[#] (kg DM d^{-1})	0.95	1.31	0.079	0.004
Live-weight gain[#] (LWG; g d^{-1})	110	159	35.5	0.216
Concentrate conversion efficiency[#] (g LWG (g DM intake)$^{-1}$)	0.113	0.118	0.0337	0.885
Condition score	3.34	3.42	0.079	0.309
Weight empty (kg)	34.8	35.0	0.68	0.724
Cold carcass weight (kg)	17.0	17.5	0.59	0.390
Killing-out ratio	0.487	0.499	0.0100	0.263
Muscle depth (mm)	25.86	26.95	0.530	0.086
Back-fat depth (mm)	4.7	4.5	0.31	0.438

[#] = days 0 to 28; [$] = 6 residual df for error

Conclusions

Overall, the findings of this study show that home-grown crimped lupin-barley concentrate diets can be used to finish lambs without any detrimental effects on productivity or carcass characteristics when compared to a commercial lamb finishing diet.

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Optimal base temperature for computing growing degree-day sums to predict forage quality of mountain permanent meadow in South Tyrol

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Abstract

Knowledge of the appropriate base temperature (Tb) is needed for the analysis of meteorological effects on plant phenology using growing degree-days. However, several values of Tb have been used for mountain grassland. The main purpose of this research was to identify the optimal Tb for predicting changes in crude protein, acid detergent fibre and net energy for lactation of forage grown in mountain permanent meadows in South Tyrol (Italy). Forage samples were sequentially harvested weekly for a period of seven weeks starting from the pasture stage (15 cm growing height) at 202 environments (sites × years). To estimate the most appropriate Tb, growing degree day sum (GDD_{sum}) were computed in the time interval between one week before pasture stage and the harvest time using a range of Tb between 0 °C and 5 °C at a 0.5 °C interval and using both observed temperatures from reference weather stations and interpolated temperature. Optimal Tb were estimated on the basis of the R^2 values of the regression equations of the different quality parameters on GDD_{sum} and can be set at 0, 1.5 and 4.5 °C for the prediction of CP, NEL and ADF respectively. R^2 values exhibited in general little variation within the investigated range.

Keywords: permanent meadows, mountain environment, forage quality, base temperature, growing degree-day sum

Introduction

Growing degree-days (GDD) are widely used in agriculture to describe the phenological development of plants. During the growth period, modification of the chemical composition has an impact on the nutritional value of the plants. Degree day sum have been used to describe variation in crude protein content and cell wall components at different phenological stages of alpine species in pastures (Bovolenta *et al*., 2008). Knowledge of the right base temperature (Tb) is crucial to compute reliable GDD. However, several values of Tb have been used by different authors for grassland. Parsons *et al*. (2006) predicted fibre content of legumes at low altitudes by using a Tb of 5 °C, while Bovolenta *et al*. (2008) used a Tb value of 0 °C for high altitude pastures in the Alps. More recently, in a study across permanent meadows at altitudes up to 1100 m in Austria, Schaumberger (2011) identified optimal Tb values of 3.0 °C and 2.0 °C for the beginning of flowering of Dactylis glomerata and the time of first cut, respectively. The main purpose of this research is to estimate the optimal Tb to be used for permanent meadows in South Tyrol (Italy) covering a wide altitude range (667 to 1593 m) and to be used for predicting the change of selected forage quality traits.

Material and methods

Forage samples were collected by weekly sequential sampling for a period of seven weeks starting from the pasture stage (15 cm growing height) at 175 environments (35 experimental sites × 5 years) from 2003 to 2007, at 20 environments (5 experimental sites × 4 years) from 2009 to 2012 and at 7 environments in 2013. Details concerning sampling design and forage analyses are described in Peratoner *et al*. (2010). For each experimental site, a reference weather station (RWS) was chosen among those of the measurement network of the Hydrographic

Office of the Province Bolzano/Bozen in terms of similar altitude, limited geographical distance and missing screen effect by hills. On a daily basis, the observed temperatures of the whole measurement network were spatially interpolated by georegression at a resolution of 100 m according to Schaumberger (2011), based on the correlation of observed mean monthly temperature and elevation combined with daily residuals. R^2 and intercept of the regression equations calculated by using interpolated temperatures at the experimental site on the observed temperatures of the RWS were used to assess the suitability of the RWS. A suitable RWS was found for only 20 of the 35 experimental sites. Growing degree days (GDD) were computed according to Schaumberger (2011). Growing degree-day sums (GDD_{sum}) were calculated referring to the time interval between one week before the date at which pasture stage was attained and the harvest date, using a range of Tb between 0 °C and 5 °C at a 0.5 °C. GDD_{sum} were computed both with the temperatures observed at the RWS and the interpolated temperatures at the experimental site. Data analysis was performed with a mixed model in SAS taking into account the serial correlation due to repeated measurements within each environment and the random effects experimental site, sampling area within the experimental site, year and the interaction year × experimental site. The relationships between the dependent variables (CP, NEL and ADF) and the GDD_{sum} obtained with the different Tb were separately fitted by means of a second-degree polynomial. A first-order autoregressive covariance structure was used for modelling the serial correlation among repeated measures. CP data were square root-transformed to achieve normal distribution of the residuals and variance homogeneity. Nevertheless, visual assessment of residuals suggested mild heteroscedasticity, which will be explored in future work. A five-fold cross-validation (Hawkins *et al*. 2003) was performed for each model and the relationship between the resulting coefficient of determination and the Tb was fitted by a polynomial up to the third degree. Tb values corresponding to the stationary points of these equations (the maximum in case of the third-degree polynomial) were regarded as optimal Tb values (Tb_{opt}).

Results and discussion

Tb_{opt} were found to vary mainly depending on the quality parameter investigated and to a lesser extent on the data set used for the analysis (Figure 1).
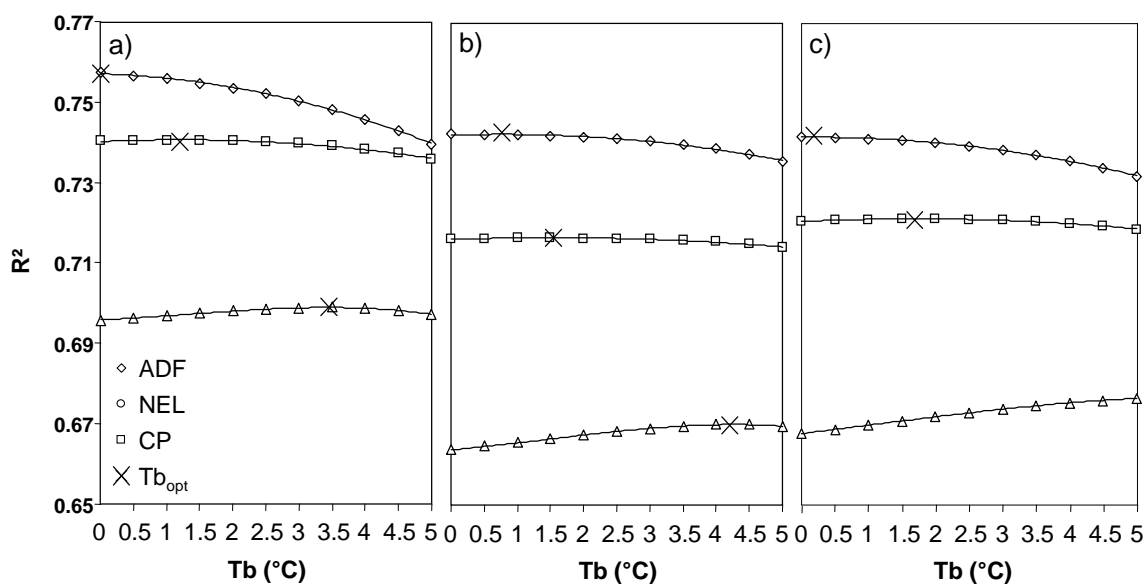

Figure 1. Polynomials fitting the determination coefficients of regression equations of different quality parameters on GDD $_{sum}$ depending on Tb and obtained based on three data sets: a) interpolated temperatures at 35 experimental sites, b) interpolated temperatures at the 20 experimental sites with an available RWS, c) observed temperatures of the 20 RWS.

All in all, R² values ranged, across the quality parameters and the different data sets, in a quite narrow interval (between 0.664 and 0.757). Moreover, changes of R² of NEL, CP and ADF depending on Tb (from 0 °C to 5 °C) were very small in the investigated temperature range. Higher coefficients of determination were consistently achieved for CP and lower coefficients of determination were observed for ADF across the different data sets. Tb_{opt} for CP was found to be located around the lowest end of the investigated temperature range, varying depending on the data set between -0.42 and 0.77 °C. The Tb_{opt} for NEL was found close to it, varying between 1.2 and 1.7 °C, while Tb_{opt} for ADF is placed at the other extreme of the temperature range, with estimated values between 3.4 and 6.2 °C (the latter is not shown in Figure 1c). For all quality parameters, the lowest Tb_{opt} were found using GDD_{sum} based on interpolated temperatures of all experimental sites. Differences of the estimated Tb_{opt} might be due to imprecision in estimating temperatures through interpolation at certain sites.

Conclusions

Small changes in Tb_{opt} for estimating changes in selected quality parameters depending on GDD_{sum} were detected and can be specifically used for this task. For the prediction of CP, NEL and ADF, Tb_{opt} of 0, 1.5 and 4.5 °C respectively seem to be adequate. On average, all Tb_{opt} values lie within the range of Tb already known from the literature.

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Added value chain of the dairy industry and its development in Central Switzerland

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Abstract

Since Switzerland (CH) is not a member of the European Community, it pursues its own agricultural policy, which imposes increased competition and an incremental approach to European conditions. In 2012, in Central-Switzerland (C-CH), 19% of the farms were in the hill region (HR) and 52% were in the mountain region (MR). The average agricultural area of the 4487 Central-Swiss dairy farms was 18 ha with a milk supply on contract of 123,500 kg/year and a productivity of 6260 kg of milk/ha. A full costs analysis showed that farms in the valley regions (VR) were more productive than farms in the HR and MR (78 kg vs. 68 kg vs. 50 kg of milk/h (working hour) respectively). The overhead and the internal overhead costs were higher in the HR and in the MR than in the VR. The cheese factories play an important role in added value, especially in remote valleys. A promising prospect under the challenging conditions (e.g. free cheese trade with the EU and the abandonment of milk quotas) is a higher efficiency of the added value chain, i.e. to reduce production costs and to produce niche products, including specialities (e.g. organic products) and brand merchandising.

Introduction

In Central-Switzerland (C-CH), situated in the northern foothills of the Alps, there are high quality grassland areas in the lowlands, as well as mountain areas of high natural value with particularly unfavourable topographical and climatic conditions. In 2008, 6.7% of the total working population (398,609 people) of C-CH was engaged in the primary sector (agriculture, forestry and fishery), 27.3% in the secondary sector (industry and handicraft) and 66.0% in the tertiary sector (services) (LUSTAT, 2012). There are significant differences between the six cantons, e.g. in Uri, 10.6% of the working population worked in the primary sector, mostly in the MR, but only 2.2% did so in Zug (in the majority of cases in the VR). Also, in Zug, 72.9% of the population is engaged in the tertiary sector. In C-CH, the dairy industry plays an important role. The aim of this study was to investigate the evolution of the dairy industry from 2000 to 2012, especially the full costs of dairy farms under different geo-economic conditions.

Materials and methods

Data on agriculture, dairy farms and factories, milk processing, consumption of cheese and food were taken from Bundesamt für Statistik (2014), the Swiss Federal Office for Agriculture (FOAG, 2014), the Swiss Milk Producer (SMP, 2014), the Central Switzerland Dairy Farmers' association (ZMP, 2012) and the GOTTLIEB DUTTWEILER INSTITUTE (GDI, 2008). Full cost accounting was conducted based on the data by Haas and Höltschi (2013), and it was performed according to VOKO-Milch + Schweine by AGRIDEA (2014).

Results and discussion

Analysis of the farm size structure: Table 1 shows that from 2000 to 2012 the total of farms was reduced by 15.6% in C-CH, whereas they decreased all over Switzerland (CH) by nearly 20%. In C-CH, the share of farms in the HR and mountain region MR was about 15% higher compared to the Swiss average. The agricultural gross value added (i.e. production value minus preparatory efforts) decreased about 8% more in C-CH than in all Switzerland.

Table 1. Farm size structure in Central-Switzerland in comparison to all Switzerland.

	Central-Switzerland (C-CH)		Switzerland (CH)	
Year [1]	2000/01	2012/13	2000/01	2012/13
Farms (all production systems), number	10659	8998	70537	56575
Therefrom of full-time farms (>75%), %	73.7	72.5	69.8	71.1
Therefrom of organic farms, %	5.5	9.4	7	10.4
Therefrom in the hill region, %	18.6	18.7	13.7	14.3
Therefrom in the mountain region, %	51.2	51.6	41.5	41.3
Gross value added, Mio. (EUR)	492	334	3910	2975
Dairy farms, number	6984	4487	38082	24103
Area (ha)[2]	15.4	18.0	19.1	23.8
Merchandised milk (kg)	72351	123500	81691	135440
Merchandised milk/ha (kg)[2]	5113	6260	4278	5595
Merchandised milk/cow (kg)[2]	4720	5805	4994	5989

[1] In 2006, the former milk quota regulation of the Swiss Confederation was abolished. [2] Data of C-CH from 2002/03.

Dairy farms: From 2000/01 to 2012/13, in the C-CH, the milk quota (since 2006 by private regulation) rose by 70% to an average of 123,500 kg of milk/farm/year, which was 9% lower than the total Swiss average. The number of C-CH dairy farms decreased by 36%. In C-CH, the merchandised milk kg per cow and per ha increased, especially the area productivity (e.g. the 3459 ZMP milk producer had on average an area of productivity of 6925 kg/ha/year. This demonstrates the high production rate of C-CH dairy farms.

Full costs accounting: The milk production per main forage area and the productivity of labour (kg of milk/h (working hour)) were very different in all regions (Table 2). More direct payments in the HR and MR could partly compensate for higher overhead (machinery, buildings and equipment) and internal overhead costs (wage entitlement) in these regions. The total agricultural income was significantly higher in the VR compared to the MR. In the MR, there was a higher return from by-products (calves or cull cows) compared to the VR and HR.

Compared to the average of all production systems, full-time grazing systems in the VR, HR and MR showed a higher labour income (€/h) of 23%, 6% and 4% respectively. In the VR, organic farms showed a 39% higher labour income than usual farms (e.g. with an ecological performance certificate) and farms with hay (for cheese factories) conservation systems showed a 15% higher labour income than farms with silage. In the HR, organic farms (+25%) and farms with hay (+6%) generated a higher labour income. In the MR, organic farms had a higher labour income (+13%) while farms with hay conservation systems had nearly the same income (+1%).

Milk processing and cheese factories: In 2012, within CH, 88% of the total amount of milk was processed (therefrom 48% went to cheese, 19% to butter, 9% to cream and 23 % to other products (milk preserves, yoghurt, *etc*.)). In C-CH the two largest creameries processed 44% (i.e. 1537 Mio. kg/year) of the total amount of merchandised milk. But the number of small cheese factory co-operatives (average milk processed of Mio. 2.28 kg/year) decreased from 139 in 2000 to 50 in 2012. In 2012, within C-CH, there were 26 Emmental cheese factories (75% fewer than in 2000) and 16 Sbrinz cheese factories (27% fewer than 2000), 2 Le Gruyère cheese factories (33% fewer than in 2000) and 8 semi-hard and soft cheese factories (11% fewer than in 2000). This immense decrease of hard cheese factories was due to changes in the eating and consumption behaviour of soft and semi-hard cheese, the abandonment or merger of

uncompetitive small units, and the farmers changing their conservation system from hay to silage or abandoning milk production in favour beef production.

Limitations: The farms for the full costs analysis were not randomly selected. These data were recorded from farms whose managers were attending further vocational training in dairy farming. We suspect that these farms have a higher performance rate than average ones.

Table 2. Full costs analysis of dairy farms (mean ± SD) in valley region (VR), hill region (HR) and mountain region (MR) in Switzerland (without group farming).

Book keeping data from 2009 to 2012	VR		HR		MR		Significance[1]		
About half of these farms are in C-CH	174 dairy farms		68 dairy farms		105 dairy farms		VR v	VR v	HR v
Costs and income are per kg of milk	Mean	±SD	Mean	±SD	Mean	±SD	HR	MR	MR
Agricultural area (ha)	31	15	25	12	25	14	**	**	
Number of cows (cows/dairy farm)	35	16	29	13	22	10	*	**	**
Merchandised milk (kg year^{-1})	257314	128378	203120	111513	125885	74028	**	**	**
Milk production/cow / year (kg)	7800	1074	7307	1110	6393	1117	**	**	**
Milk/main forage area (kg ha^{-1})	12800	3924	10601	3285	6666	3255	**	**	**
Labour productivity (kg of milk h[-1])	78	23	68	24	50	15	**	**	**
Direct costs (Cent kg^{-1})[2]	-22	5.7	-22	5.4	-23	6.5			
Therefrom concentrate (Cent kg^{-1})	-9	3.2	-9	3.1	-9	3.9			
Overhead costs (Cent kg^{-1})	-32	8.2	-36	11.9	-46	15.0	**	**	**
Internal overhead costs (Cent kg^{-1})	-26	11.5	-31	13.5	-51	23.0	*	**	**
Full costs (Cent kg^{-1})	-80	13.1	-88	22.7	-119	33.5	**	**	**
Milk price (Cent kg^{-1})	51	7.0	52	7.5	52	8.2			
Direct payment (Cent kg^{-1})	17	7.0	23	7.1	39	15.2	**	**	**
Profit/loss (Cent kg^{-1})	-12	11.2	-13	12.8	-28	22.5		**	**
Labour income h[-1] (€)	11	8.1	13	5.8	11	5.4			**
Agriculture income dairy farming (€)	39014	28585	40914	27888	38516	26492			
Therefrom by-products (€)[3]	5559	5519	6738	5591	10967	9733		**	**
Agricultural income total (€)	63200	41412	58019	40185	48750	32715		**	

[1] * = $P < 0.05\%$; ** = $P < 0.01$. [2] Actual currency: 1 CHF = 0.818034 EUR. [3] Calves and cull cow returns.

Conclusions

From 2000 to 2012, in C-CH, the number of dairy farms and their agricultural gross value added decreased. Therefore, jobs providing additional income are essential in such fringe areas. With regard to the high production costs of the dairy farms, small to medium sized farms, especially, are under considerable strain. The productivity of the dairy farms in all regions must improve due to rising competition, e.g. co-operation, particularly with respect to
farm mechanisation and the use of buildings, becomes very important. With a rising demand for meat worldwide, the by-products will become increasingly important.

In mountain regions, cheese factories maintain added value. If cheese factories can develop, apart from improving their efficiency, milk-based specialities and then merchandise them in the European marketplace, they may generate a higher-than-average milk price. Such a milk price would partially compensate for higher production costs in mountain areas.

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Economics of grazing

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Abstract

This study provides insight into the economics of grazing on modern Dutch dairy farms and shows how the yield from grazing can be improved. Model calculations show that grazing is financially attractive if the cows eat sufficient amounts of fresh pasture grass (> 600 kg DM cow^{-1} yr^{-1}). If the intake of fresh grass is very low, grazing is less profitable than summer feeding. Statistical analysis of the actual financial results of Dutch commercial dairy farms confirmed the positive effect of grazing. The model calculations showed a larger financial benefit of grazing than actually achieved in practice. The differences were examined together with extension services. Feasible possibilities for increasing the benefits of grazing were studied. It was concluded that commercial dairy farms often do not take full advantage of grazing because their operational management is not optimally adapted to grazing and maximum fresh grass intake. Grazing strategies must be implemented consistently for optimal financial benefit. The intake of fresh grass must be high, but at least more than 600 kg DM cow^{-1} yr^{-1}.

Keywords: economy, grazing, management

Introduction

In northwest Europe, grazing is a matter of public concern (e.g. Van den Pol-van Dasselaar *et al*., 2008). The main reasons for this are animal welfare, biodiversity and the positive image of grazing. Grazing has both positive and negative effects on the environment, the most obvious being nutrient loss. In general, grazing is economically attractive for farmers. However, an average situation will not be applicable to all farms. Certain conditions may be less favourable for grazing. Van den Pol-van Dasselaar *et al*. (2010) showed for the Netherlands that, for situations with automatic milking systems, large herds and high milk yields per cow, the farmer's income remained the highest for grazing. However, in situations with more than 10 dairy cows ha^{-1} grazing surface, it was not profitable to practise grazing. There was a strong relationship between intake of grass in pasture, on a typical farm, and the difference in income between grazing and no grazing. The more grass the cows ate in the pasture, the larger the income profit from grazing compared to no-grazing and feeding roughage. However, some time has passed since this study. Since then, (environmental) policy has changed, farms developed and the corresponding increases in scale have continued. Farming systems have become increasingly intensive, and more and more farms have started to use automatic milking systems. This study therefore focuses on the economics of grazing on modern Dutch dairy farms and the possibilities to improve the financial benefits from grazing on these farms.

Materials and methods

Gross margins were used to calculate the economics of grazing in the Netherlands for the near future (2015-2020) using the model DairyWise (Schils *et al*., 2007). The DairyWise model is an empirical model that simulates technical, environmental and financial processes on a dairy farm. The central component is the FeedSupply model that balances the herd requirements, as generated by the DairyHerd model, and the supply of home-grown feeds, as generated by the crop models for grassland and maize silage. The GrassGrowth model predicts the daily rate of dry matter accumulation of grass, including several feed quality parameters. The final output is a farm plan describing all material and nutrient flows and the consequences on the environment and economy. As part of the calculation, the expected developments and trends with respect to

policy, increasing scale and automation were taken into account. Production intensities ranging from 15,000 to 30,000 kg milk ha^{-1} and average milk productions of 8,500 kg milk cow^{-1} yr^{-1} were used. Herd size varied between 70 and 280 cows. The benefits of grazing were primarily determined by the lower subcontracting costs, higher costs for roughage, lower costs for feed concentrate and lower costs for manure disposal.

Next to modelling, data from real-life farms were used. The method data envelopment analysis (DEA) (Steeneveld *et al.*, 2012) was used for statistical analysis of farm data collected by accounting firms and advisors. The results illustrate the actual financial results of approximately 10% of all Dutch commercial dairy farms in 2011. The study used six data sets of accounting firms and advisors (Countus, DLV, DMS, Flynth, LEI and PPP-Agro Advies).

Results and discussion

The results of the gross margins calculated with DairyWise are summarized in Figure 1. It shows that grazing is financially more attractive than summer feeding if the cows eat sufficient amounts of fresh pasture grass (> 600 kg DM cow^{-1} yr^{-1}). If the intake of fresh grass is very low, grazing is not advantageous over summer feeding. In practice, the effect should be even more positive than shown in Figure 1, since the majority of the Dutch farmers currently receive a grazing premium of 0.5 euro for each 100 kg milk if they graze their dairy cattle for at least 120 days yr^{-1} and 6 h d^{-1}. This additional income was not taken into account in Figure 1.

When analysing the financial records of commercial dairy farms, large differences were found between farms regarding efficiency and gross operating profit. On average, grazing resulted in more efficient operational management and a higher gross margin. However, these positive results declined in relation to increasing farm size. In 2011 the transition point was, on average, a farm size of about 90 dairy cows. If grazing was combined with automatic milking, much of the financial advantage of grazing disappeared. In 2011, the majority of dairy farms did not have the option of receiving a grazing premium. Today, however, most dairy companies have implemented the grazing premium. The current grazing premium would have made the transition point move up to a farm size of approximately 130-140 cows. Unfortunately, the actual grass intake on the commercial dairy farms was not known. Therefore, it was not possible to relate the grass intake to the farm income. The category 'grazing farms' included both farms with very low grass intake and farms with full grazing. The results as shown in Figure 1 implicate that knowledge on the actual grass intake would have led to a more detailed insight in the economics of grazing on commercial dairy farms.

In the model calculations, the financial benefits of grazing were larger than those actually achieved in practice in 2011. To understand these differences more clearly, the situation in practice was examined along with the feasible possibilities for increasing the benefits of grazing. This exercise was done together with extension services. It was concluded that farms which graze their livestock do not take full advantage because their operational management is not optimally adapted to grazing and optimal grass intake. The intake of fresh pasture grass by their cattle is inadequate. Dairy farmers can improve the financial yield from grazing with relatively simple measures, like providing less supplementary feed, and by starting the grazing early in the season and ending late in autumn. By using the land parcels closest to the barn for grazing, and growing silage maize further away, or providing access to pasture on the other side of the road, the fresh grass intake can possibly be increased even further. Finally, a grazing strategy must be implemented consistently for optimal financial benefit. To benefit financially, the intake of fresh grass must be sufficient, at least more than 600 kg DM cow^{-1} yr^{-1}.
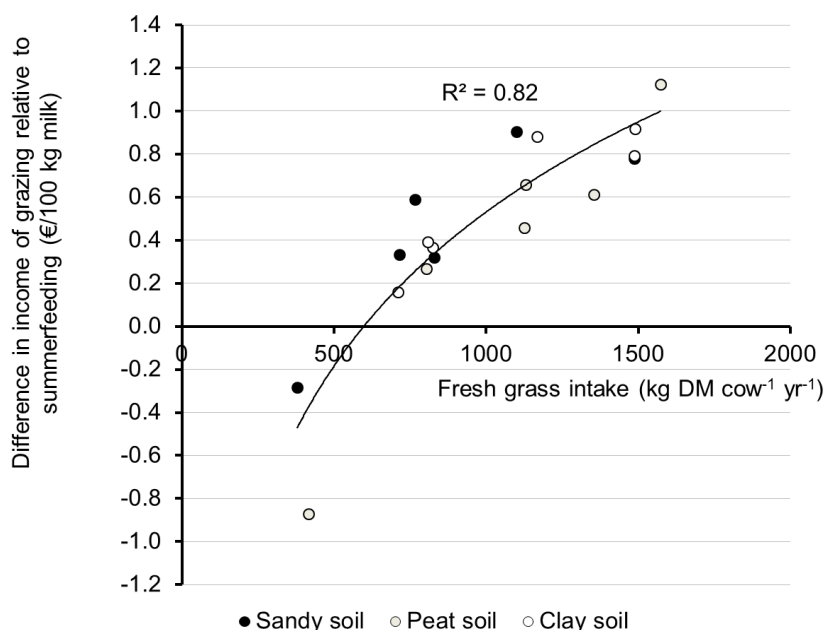
Figure 1. Income with grazing minus income with summer feeding relative to the amount of fresh grass intake in kg dry matter (DM) cow^{-1} yr^{-1} for three soil types.

Conclusion

In many cases, grazing offers financial benefits, on larger farms and also on automated farms. However, this does not apply to every dairy farm. When transposing research results into practice, the context of the individual dairy farm must always be taken into account. The current study has provided possibilities for improving the financial yield on farms that choose to graze their cattle. The results show that the fresh grass intake of the cattle is crucial for financially beneficial grazing.

Acknowledgements

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Does early spring grazing stimulate spring grass production?

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Abstract

Early spring grazing may stimulate grass growth in spring by increasing grass root exudation, which enhances mineralization. The objective of this study was to investigate the net effect of early spring grazing on spring grass production. Therefore, we conducted two field experiments on production grasslands. Simulated early spring grazing negatively affected the yield of the first cut: compared to ungrazed plots on sandy soil and a tendency on clay soil, dry matter yield of early-grazed plots was reduced by 20% (sand, *P*=0.009) and 12% (clay, *P*=0.062), respectively. These differences were partly compensated in later cuts. Despite the negative effect on grass yield of the first cut, early grazing positively affected the crude protein (CP) content of the grass in the first cut on sandy soil, but only in the plots that had not been fertilized with CAN. The stimulating effect of early spring grazing on soil nutrient mineralization appears to be too small to compensate for the negative effects of early grazing on grass leaf area and photosynthesis capacity.

Keywords: Grazing, root exudates, mineralization, spring production

Introduction

For dairy farmers the key question in spring is how to get the grass growing as soon as possible. The easiest method is to add nitrogen fertilizer, but due to stricter fertilizer regulations farmers have to depend more and more on soil nutrient mineralization. This process starts when the soil warms up in spring, and can be stimulated by mechanical soil aeration and lime additions. Furthermore, according to the experience of various dairy farmers, grass growth may also be stimulated by 'early spring grazing', a method in which grasslands are grazed in early spring for a short period of time. A possible explanation for this effect is that grazing leads to increased root exudation, which in turn triggers mineralization (Hamilton *et al.*, 2008). However, it is not known whether this mechanism also works in spring when soil temperatures are still low. Furthermore, it is unclear whether the positive effects of grazing on mineralization could outweigh the negative effect of (early) grazing on photosynthetic leaf area and thus growth rate. Therefore, the objective of this study was to investigate the net effect of early spring grazing on spring grass production.

Materials and methods

In 2013 we conducted two field experiments on production grasslands: one on a shallow sandy soil in the south of the Netherlands, and one on a clay soil in the north. All plots in both experiments were fertilized with cattle slurry at 25 m^3 ha^{-1} in mid-March. The experiment on sandy soil consisted of four treatments with six replicate plots: early spring grazing with, and without, the addition of artificial fertilizer; artificial fertilizer only; and a control. To this end, on 19 April half of the plots were mowed to ±4cm with a lawn mower to simulate early spring grazing. On the same day, half of the mowed and unmowed plots received 50 kg N ha^{-1} of calcium ammonium nitrate (CAN). The experiment on clay soil consisted of three treatments with five replicate plots: early spring grazing on 19 April (lawn mower treatment, see above); artificial fertilizer (50 kg N ha^{-1} of CAN); and a control. The effect of early spring grazing was measured in the first two cuts. The plots on sandy soil were cut on 19 May and 8 July; and on clay soil on 24 May and 2 July. Grass production of each plot was determined by mowing a

strip of 0.84 m × 5 m (sandy soil) or 1.50 m × 10 m (clay soil). Half of the yield from mowing the plots at t=0 to simulate early grazing was added to the yield of the first cut. After weighing the fresh biomass, a sub-sample was analysed for dry matter and crude protein (CP) content. Results were analysed for significance by ANOVA and Tukey's test.

Results

Simulated early spring grazing negatively affected the yield of the first cut compared to ungrazed plots on sandy soil, and there was a tendency on clay soil: dry matter yield of early-grazed plots was reduced by 20% (sand, $P=0.009$) and 12% (clay, $P=0.062$), respectively (Figure 1). These losses were partly compensated by the yields of later cuts: on sandy soil, total grass yield of the first and second cut of early-grazed plots was only 8% lower than of ungrazed plots; on clay soil it was only 5% lower. This 'catch-up' effect is likely to be due to higher re-growth rates after the first cut in the early-grazed plots, compared with the ungrazed plots where the first cut was heavier because no simulated grazing had taken place.
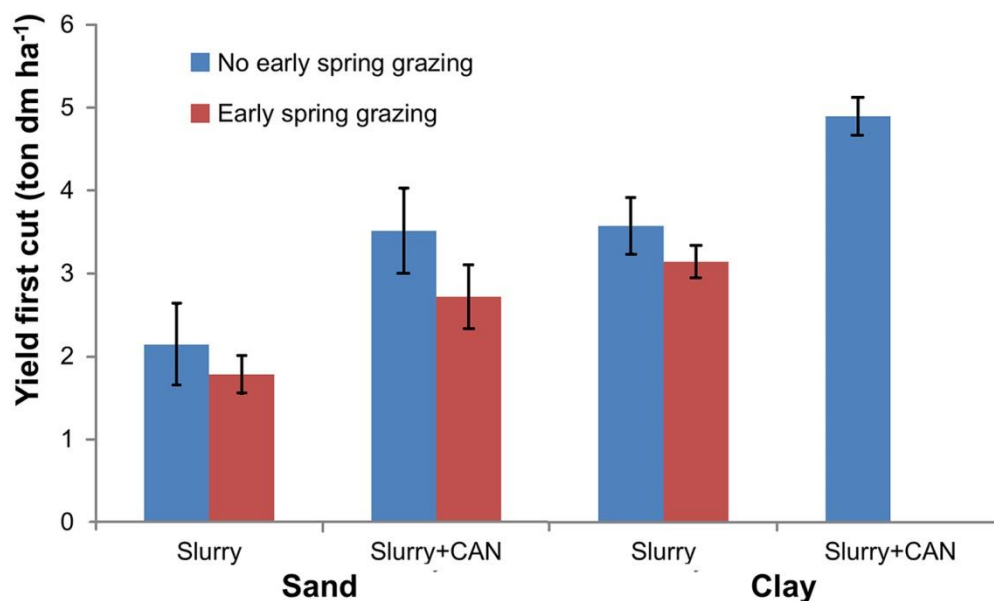

Figure 1. The effect of early spring grazing on dry matter yield of the first cut (sandy soil: 19 May; clay soil: 24 May) in plots with and without artificial fertilizer addition. Note that the yield from early-grazed plots includes 50% of the yield from mowing these plots at t=0 to simulate early grazing. Slurry=cattle slurry (25 m^3 ha^{-1}, applied to all plots including the control), CAN (50 kg N ha^{-1}). Error bars represent 2×standard deviation.

Despite the negative effect on grass yield of the first cut, early grazing positively affected the CP content of the grass in the first cut on sandy soil, but only in the plots that had not been fertilized with CAN. On sandy soil, early grazing increased the CP content of the first cut by 12%, to 151 g kg dm^{-1} compared to 135 g kg dm^{-1} in ungrazed plots (Figure 2, $P=0.001$). On clay soil the difference of 6% was not significant: 144 g kg dm^{-1} in the early grazing plots compared to 136 g kg dm^{-1} in ungrazed plots. The results on sandy soil suggest that early spring grazing promotes feed quality. This can be considered a positive effect of early spring grazing, particularly if feed quality is an issue (for example, in the case of delayed cuts, and in organic dairy pastures with low clover density). Due to the higher relative CP content of early-grazed grassland, the total nitrogen yield of early-grazed versus ungrazed plots was not significant different, in both sandy and clay soil experiments.
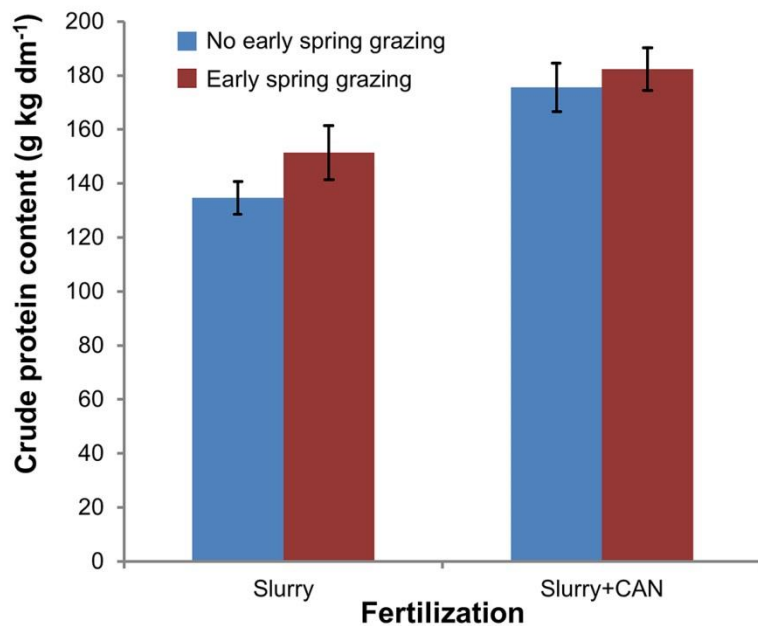
Figure 2. The effect of early spring grazing on CP content of the first cut (19 May) in plots with and without artificial fertilizer, in the sandy soil experiment. Slurry=cattle slurry (25 m^3 ha^{-1}, applied to all plots including the control), CAN (50 kg N ha^{-1}). Error bars represent 2×standard deviation.

Discussion

Contrary to expectations, early spring grazing was not found to 'kick-start' grass production. It is possible that the timing of the first cut was too early to detect a positive effect but, even if that were the case, our results show that the effect of artificial fertilizer on dry matter yield and CP content is likely to be much greater than any effect of early grazing (Figures 1, 2). Thus, it appears that early spring grazing provides no real alternative to artificial fertilizer for encouraging early grass growth in spring. Other methods still worth exploring are mechanical soil aeration and liming, both of which should stimulate soil biotic activity, nutrient mineralization, and hence grass growth.

Conclusions

Early spring grazing leads to a higher crude protein content in grass on sandy soil.
The stimulating effect of early spring grazing on soil nutrient mineralization appears to be too small to compensate the negative effects of early grazing on photosynthesis capacity.
The negative effect of early grazing on dry matter yield of the first cut is compensated in later cuts.

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Use of milk fatty acid composition to authenticate cow diets

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Abstract

The aim of this work was to authenticate cow feeding system from fatty acid (FA) concentrations of cow milk. The milk samples and records of their related farming practices were collected on 156 commercial farms in the lowland area of the Piedmont Region (North-West Italy). Milk samples were analysed for FA composition by gas-chromatography. To define the main cow feeding systems, a hierarchical cluster analysis was performed on the proportion of different feeds in the cow diets. Samples were classified into two main groups, according to whether high or low forage-to-concentrate ratio (FC): HFC and LFC, representative of extensive and intensive farming systems. Each group was divided into two subgroups: the HFC into FP and FH, in which the main forage was given as pasture, or hay, respectively; the LFC into CR or CC, if concentrates were given as raw materials or as pre-formed commercial concentrate mix, respectively. Linear discriminant analysis (LDA) was performed on milk FA concentration to authenticate the FC groups, and correctly classified 95.5% of samples in cross-validation. Promising results were found in the authentication of FP and FH subgroups (15.4% of error in cross-validation). LDA was not able to distinguish between CR and CC milk.

Keywords: milk fatty acid, feeding system, authentication

Introduction

Several researches have been made to identify reliable authentication methods for dairy products (Prache *et al.*, 2005). Among the different potential analytical tools, milk fatty acid (FA) composition was the more effective method to achieve precise information about cow feeding (Engel *et al.*, 2007). Differences in milk FA composition according to cow diet have been shown by several authors (Chilliard *et al.*, 2007; Coppa *et al.*, 2013). However, only few studies have tested the potential of bulk-milk FA profile to authenticate the cow diets adopted by commercial farms. The aim of this work was to authenticate cow diets from FA concentrations of bulk milk collected on different commercial farms in North-West Italy.

Materials and methods

Bulk cow-milk samples and information on their related farming practices were collected from 156 commercial farms located in the lowland area of Piedmont Region, in North-West Italy, across spring and summer 2013. During each milk-sampling, cow diet composition was recorded through a detailed on-farm survey. Each milk sample was analysed for FA composition by gas-chromatography. To define the main cow feeding systems, a hierarchical cluster analysis was performed on survey data on the proportion of different feeds in the cow diet. Linear discriminant analysis (LDA) was performed on milk FA concentration to authenticate the groups derived from the cluster analysis.

Results and discussion

Milk samples were classified into two main groups by the cluster analysis, according to whther they had a high or low forage-to-concentrate ratio (FC): HFC and LFC. Each group was divided into two subgroups: the HFC into FP and FH, in which the main forage source was either pasture or hay, respectively; the LFC into CR or CC, in which the concentrates were given as raw materials or as pre-formed commercial concentrate mix, respectively. The milk yield was about

9.3 kg/cow per day for HFC, and 25.6 kg/cow per day for LFC, and 8.3, 10.2, 25.0 and 26.1 kg/cow per day for FP, FH, CR and CC, respectively. The average diet composition of HFC and LFC groups, and of FP, FH, CR and CC subgroups, are given in Table 1. The HFC feeding system was representative of extensive farming systems, with cow diets based on herbage-derived forages, with high proportions of forage. The LFC feeding system was representative of intensive farming systems, which are based on cow diets of maize silage, with a high concentrate proportion. The FP and FH subsystems of HFC represented the seasonality of herbage-based forage production systems: grazing pasture when fresh herbage is available, and/or conserved forage (mainly hay) for the periods of the year in which fresh herbage is unavailable. The CR and CC subgroups of LFC represented different farming strategies, involving a cheaper, but more complex (CR), or a easier, but more expensive, (CC) concentrate supplementation and management (Borreani *et al*., 2013).

Table 1. Average diet composition of the high (FC+) or low (FC-) forage-to-concentrate ratio groups, and of their two subgroups: main forages given as pasture (FP), or as hay (FH), and concentrates given as raw materials (CR) or as pre-formed commercial concentrate mix (CM).

Feedings (% of diet DM1)	HFC	LFC	HFC		LFC	
			FP	FH	CR	CC
Maize silage	12.1	38.6	6.5	17.2	38.6	38.6
Grass or legume silage	0.0	1.1	0.0	0.0	1.0	1.3
Hay	40.9	18.2	14.6	64.9	19.0	17.7
Fresh herbage	34.7	0.4	67.4	4.9	1.0	0.0
Soybean meal	0.2	4.6	0.4	0.1	9.7	0.6
Maize or barley flour	2.0	13.4	2.3	1.7	16.2	11.2
Commercial concentrate mix	6.6	17.0	4.1	8.9	4.2	27.4
Total forages	88.1	60.6	89.3	87.0	62.3	59.3
Total concentrates	11.9	39.4	10.7	13.0	37.7	40.7

The results of linear disciminant analysis based on milk FA composition between HFC and LFC and, within each group, between the subgroups, are given in Table 2. The LDA between HFC and LFC correctly classified the 95.5% of samples in cross-validation. The milk of HFC farms was characterized by high concentration of n-3 FA (C18:3n-3, C20:5n-3, C22:5n-3), conjugated linoleic acid (CLA)c9t11, CLAc9c11, C18:2t11c15, branched chain FA (BCFA), C17:0, C17:1c9, and C15:0. The LFC milk had higher concentrations of even-chain saturated FA (ECSFA), n-6 FA, C18:1t12, C18:2c9t12+c9t14, C18:1t6/7/8, C18:1t9, and C18:1t10. These FC+FA profiles are similar to those reported in the literature for milk derived from fresh herbage- or hay-based diets, and those of FP, to milk derived from low forage-based diet (Vlaemink *et al*., 2006; Chilliard *et al*., 2007).The results of the LDA performed between FP and FH groups were promising, especially considering the low number of samples, but were less reliable than those between the two main groups (15.4% of error in cross-validation). The milk of FP farms was characterized by higher concentrations of C18:1t11, C18:2t11c15, C18:1t9, C18:1t13, C18:1t5, C18:1c10, C18:2c9t13, CLAc9t11, CLAc9c11 and C18:0, compared to FH milk. The milk of the FH group showed high concentrations of ECSFA compared to FP milk. The effect of pasture feeding on milk FA composition is well known (Chilliard *et al*., 2007; Coppa *et al*., 2013) and in agreement with our results. The LDA was not able to distinguish between CR and CC milk: only 66.3% of samples were correctly classified in cross-validation. However, the CR milk had higher concentrations of BCFA, n-3 FA, C18:2t11c15 and C17:0, than the CC milk. The CC milk had higher concentrations of n-6 FA, and of several C18:1 isomers different from C18:1t11.

Table 2. Results of linear discriminant analyses based on milk FA composition between the high (HFC) or low (LFC) forage-to-concentrate ratio groups, and within each group, between the two subgroups: main forages given ad pasture (FP), or as hay (FH), and concentrates given as raw materials (CR) or as pre-formed commercial concentrate mix (CM).

Feeding system[1]	Number of milk samples	Calibration		Cross-validation	
		Samples misclassified	Correct classifications (%)	Samples misclassified	Correct classifications (%)
HFC *vs.* LFC	157	4	97.5	7	95.5
FP *vs.* FH	65	4	93.8	10	84.6
CR *vs.* CC	92	16	82.6	31	66.3

Conclusion

In the Piedmont Region, two main feeding systems were identified, corresponding to intensive and extensive farming systems. The milk FA composition was successfully used to authenticate milk derived from these two systems. The milk FA composition gave also promising results in the authentication of fresh herbage- or hay-based diets within the extensive systems. It was not, however, possible to authenticate the concentrate supplementation strategy within the extensive farming systems.

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Potential lipid markers of plant species from grasslands to authenticate mountain dairy foods

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Abstract

In different mountain areas of Europe milk and cheese are traditional foods, and their survival in the future will depend on the sustainability of the mountain grazing livestock. High quality milk and cheese production, together with adequate tools to protect and authenticate these traditional foods, are important issues to ensure the continuity of the mountain grazing systems. Plant lipids can be potential markers of products obtained from grass-fed animals due to the transfer of these compounds directly from pasture to foods or because they are precursors for other specific lipid compounds generated by the animal metabolism. In this study, fatty acids (FAs), tocopherols, carotenoids and terpenoids of the main plant species present in a grazing area of the Cantabrian Mountains were analysed. Some of the major potential lipid markers of dairy products obtained from mountain pasture-fed animals could be α-linolenic and linoleic acids, α-tocopherol and α-tocotrienol, lutein, β-carotene, α-pinene, β-ionone, β-thujene, α-copaene, γ-cadinene and β-cubebene.

Keywords: mountain pasture, fatty acids, tocopherols, carotenoids, terpenoids

Introduction

Different types of lipids have been proposed as potential tracers for milk and cheese from grazing ruminants. Some of these compounds can come directly from pasture, whereas other compounds are derived from animal metabolism (Prache *et al*., 2005). The fatty acid (FA) composition of dairy products is primarily affected by the animals' diet, and it has been reported that grazing livestock increases the content in the milk of some beneficial FAs, like vaccenic and rumenic acids. Dairy products from grass-fed ruminants are also enriched in polyunsaturated FAs (PUFAs) such as linoleic and linolenic, which are the major PUFAs in fresh grass (Cabbidu *et al*., 2005). Tocopherols (vitamin E) and carotenoids, such as lutein and β-carotene, can also be directly transferred from fresh grass into milk, but this depends on the animal species and other nutritional factors (Morand-Fehr *et al*., 2007). Animal metabolism will also affect the conversion of β-carotene to retinol (vitamin A), and therefore, its accumulation in milk will depend largely on the ruminant specie (Noziére *et al*., 2006). Terpenoids originate from the secondary metabolism of plants and they can be rapidly transferred into milk (Viallon *et al*., 2000). This paper summarises the composition of main FAs, tocopherols, carotenoids and terpenoids of the most abundant plant species found in Cantabrian mountain pastures.

Materials and methods

Thirteen major plant species were sampled in the second week of May and June (2012) in pastures of the Aralar Natural Park (42° 59' 48" N 2° 06' 51" W) in northern Spain. Grass samples were lyophilized before analysis. FAs were extracted and analysed by GC-FID according to a method adapted from Chávarri *et al*. (1997). Tocopherols and carotenoids were simultaneously extracted (Cardinault *et al*., 2008). Then, tocopherols were analysed by normal-

phase HPLC with a fluorescence detector (Panfili *et al.*, 1994), while carotenoids were determined by reverse-phase HPLC coupled with a diode-array detector (Kimura *et al.*, 2007). Grass terpenoids were analysed by SPME-GC-MS using a method adapted from Abilleira *et al.* (2010). Mean values of the aforementioned lipid compounds were calculated for each of the botanical families present in the pastures. Student's *t* test was applied to study significant differences ($P \leq 0.05$) in the lipid composition of botanical families between May and June.

Results and discussion

Around thirty FAs were detected in the studied plant species and PUFAs represented the highest content in all the botanical families. α-Linolenic content was the largest in *Poaceae* (52%), *Fabaceae* (43%), *Lamiaceae* (39%) and *Rosaceae* (34%). On the other hand, linoleic content was the largest in *Asteraceae* (44%) and *Juncaceae* (38%) (Table 1).

Table 1. Major potential lipid markers from the main botanical families collected in summer (May and June) in Cantabrian mountain pastures. Mean values are expressed as mg/100 of dry matter except for terpenoids (peak area values relative to that of an internal standard).

Lipid compound	Monocotyledon families		Dicotyledon families			
	Poaceae[a] (s=4)	*Juncaceae*[b] (s=1)	*Fabaceae*[a] (s=4)	*Asteraceae*[a] (s=2)	*Rosaceae*[a] (s=1)	*Lamiaceae*[c] (s=1)
α-linolenic acid	*507	156	526	*337	*232	315
linoleic acid	209	468	331	566	*195	230
α-tocopherol	2.09	0.637	2.64	1.94	*2.62	6.86
α-tocotrienol	0.152	nd	0.127	0.334	nd	nd
lutein	13.9	4.79	11.8	*7.57	*7.72	6.82
β-carotene	*2.95	1.62	2.38	*1.09	*1.53	1.83
α-pinene	16.7	10.9	52.4	432	18.8	$113 \cdot 10^2$
α-phellandrene	14.8	10.9	25.3	419	*19.5	$448 \cdot 10^1$
β-thujene	15.0	11.2	30.5	$129 \cdot 10^1$	*19.5	$395 \cdot 10^1$
β-ionone	190	62.8	*128	*125	145	156
isoeugenol	27.0	18.6	68.9	117	40.3	$723 \cdot 10^1$
α-copaene	*124	*104	165	333	*89.0	249
β-cubebene	*142	102	226	458	*50.3	399
β-caryophyllene	*142	68.8	179	304	*107	$172 \cdot 10^1$
δ-cadinene	38.3	23.4	72.5	$101 \cdot 10^1$	*67.1	824
γ-cadinene	152	90.7	*162	861	*188	$479 \cdot 10^1$

[a]Samples collected in May and June; [b]samples collected only in May; [c]samples collected only in June; s, number of plant species collected;
*statistically significant ($P \leq 0.05$) differences between months.

These differences could be mainly related to genetic factors, phenological stage of plants or climatological variables. In this respect, the most remarkable differences were the higher content of α-linolenic in June than in May for *Poaceae*, *Rosaceae* and *Asteraceae* families. Seven tocols were detected in the plant species but α-tocopherol was the largest ($\geq$ 70%) in all botanical families. Among other minor tocols, α-tocotrienol was found to be higher than 5% in *Poaceae*, *Fabaceae* and *Asteraceae* (Table 1). For *Rosaceae*, only α-tocopherol content was higher in June than in May. Seven xanthophylls and four carotenes were identified in the plant species but many peaks with carotenoid spectra, depending on the plant species, remained unknown. Despite the differences observed in carotenoid composition, lutein was the major carotenoid in all botanical families, ranging from around 50 to 75% of the total identified carotenoids, while the content of β-carotene ranged from around 12% in *Poaceae* and *Fabaceae* to 26% in *Juncaceae* (Table 1). Higher content of lutein and β-carotene was found in June than in May when significant differences were observed in botanical families.

More than seventy terpenoids were found in the plant species from the mountain pasture, most were monoterpenes (53%) followed by sesquiterpenes (43%) and chemically irregular terpenes (4%). Significant differences between botanical families were found in the terpenoid content

and *Lamiaceae* appeared to be the botanical family with the highest content, of both mono- and sesquiterpenes (Table 1). The most abundant terpenoids found in *Lamiaceae*, *Poaceae* and *Asteraceae* were α-pinene (21%), β-ionone (9%) and β-thujene (9%), respectively, whereas the most abundant terpenoids found in *Rosaceae*, *Juncaceae* and *Fabaceae* were γ-cadinene (9%), α-copaene (8%) and β-cubebene (8%), respectively. Changes in the content of terpenoid composition between May and June were dependant on botanical families and individual compounds.

Conclusion

The characteristic lipid profile of major plant species of Cantabrian mountain pastures could provide useful information to authenticate dairy products obtained from grazing animals. Some of the major potential lipid markers from plants could be α-linolenic and linoleic acids, together with α-tocopherol, α-tocotrienol, lutein, β-carotene, α-pinene, β-ionone, β-thujene, α-copaene, γ-cadinene and β-cubebene.

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Is phytanic acid a suitable marker for authentication of milk and dairy products from grass-fed cows or organic farming systems?

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Abstract

Cow milk samples were collected from herds of 30 Dutch farms and analysed by gas chromatography-mass spectrometry for phytanic acid (PHY) and its diastereomers SRR and RRR to test the hypothesis that PHY could be a suitable marker for authentification of milk from grass-fed cows. The samples differed in the proportion of fresh grass in the cows' daily dry matter intake (0 to 94%). Grass was either fed indoors or grazed (during daytime, or day and night). Of the latter category, three farms had an organic and three a biodynamic farming system. PHY concentrations were not significantly higher in organic/biodynamic milk compared with conventional milk, nor were they correlated with the proportion or amount of fresh grass in the diet. The proportion of RRR in total PHY was positively correlated with the proportion of fresh grass in the diet. These results indicate that, in contrast to our hypothesis, PHY content is not a suitable indicators of pasture grazing or organic/biodynamic farming, while the proportion of diastereomers of PHY may be useful as such.

Keywords: milk, authentication, grass-fed dairy, organic, phytanic acid, grazing, cow diet

Introduction

In the Netherlands, the label 'Weidemelk' ('pasture milk') was introduced in 2012 for milk from cows that are kept at pasture at least four months per year and 6 hours per day (Elgersma, 2012). Phytanic acid (PHY) is a branched-chain fatty acid, produced by bacteria from enzymatic degradation of chlorophyll in the rumen. PHY cannot be synthesized *de novo* by mammals, and thus its presence in milk and animal-derived products depends exclusively on feed, especially on green materials, i.e. grass and forage which contain chlorophyll. Since the proportion of grass in animal rations often is higher in organically raised cattle, PHY has been proposed as a marker for for pasture-fed milk and dairy products, as well as for organic dairy products (Vetter and Schroeder, 2010). We hypothesized this might be used in Dutch milk to authenticate 'weidemelk' and organic milk.

Along with PHY content, the ratio between the two diastereomers of PHY, i.e. 3R,7R,11R-PHY (RRR) and 3S,7R,11R-PHY (SRR), which form upon biohydrogenation of the phytol double bond, has been shown to largely vary according to animal diet and has also been proposed for the authentication of pasture grazing and organic feeding (Schroeder and Vetter, 2011; Baars *et al.*, 2012). However, some recent findings indicate that total PHY content is not necessarily directly correlated with the intake of green feed, being linked in a more complex way to the whole diet (Che *et al.*, 2013). Capuano *et al.* (2014) reported that PHY content was not a suitable indicator of pasture grazing or organic/biodynamic farming system. The aim of this investigation was to further explore the effect of fresh grass in cows' diet and of organic/biodynamic farming systems on the share of RRR in total PHY in raw cow farm milk in the Netherlands and discuss implications for the verification of farm management system.

Materials and methods

Thirty farm-tank samples of raw milk were collected from 30 Dutch farms between 7 and 27 July 2011. Farm management and ration information of herds (amounts and proportions of feeds

in total DM intake) from the week of sampling was obtained by means of questionnaires and interviews. Six farms did not feed any fresh grass to the cows (group NG). The remaining 24 farms fed fresh grass in amounts ranging from 0.36 to 0.94 of total DM intake. Four of those farms fed cut grass indoors (group GI) whereas the remaining 20 farms practised grazing at pasture either during daytime only (8 farms, group Pd) or day and night grazing (12 farms). Of the latter group, there were six farms with a conventional farming system (group Pd+n), and three organic (Org) and three biodynamic farms (BD); jointly named OB.

Milk fat was extracted and analysed by gas chromatography-mass spectrometry according to Capuano *et al.* (2014). Significance of differences was tested between milk samples from cows that had been (GI, Pd, Pd+n and OB) or had not been fed fresh grass (NG), and between organic/biodynamic (OB) and conventional (NG, GI, Pd and Pd+n) farming systems. Correlations were calculated between proportions of feed categories in total DM in cow diets, and the content of PHY and proportion of RRR in total PHY.

Results and discussion

The average PHY content of the milk was 1.5 mg g fat^{-1} with values ranging from 0.6 to 3.2 mg g fat^{-1} (Capuano *et al.*, 2014). The distribution of PHY was significantly different only between milk from continuous stocking (Pd+n, highest) and fresh-cut grass indoors (GI, lowest, not shown). The concentration of PHY was not higher in milk from organic / biodynamic systems than in conventional milk, nor was it correlated with the relative or absolute amount of fresh grass or any other forage or feed type or combination of feed types in the daily ration (Capuano *et al.*, 2014). These results did not confirm our hypothesis and contrast with findings reported by other authors. It has been repeatedly reported that fresh grass or grass-silage increase the PHY content in cows' plasma and milk because of the higher level of phytol (derived from chlorophyll) in fresh grass compared with maize silage, hay or concentrate (e.g., Baars *et al.*, 2012). However, the phytol content of the diet can vary among and within feeds. In grassland, the botanical composition can play a role, as well as sward structure: grazing cows select young leaves at the top of the canopy, whereas cut grass contains more older leaves and pseudostems that contain less chlorophyll (Elgersma *et al.*, 2003). Che *et al.* (2013) found a negative correlation between the amount of pasture and the level of PHY in milk, but a positive correlation between the proportion of grazed legumes in DM intake and the share of RRR in total PHY, which ranged from 0.24 in a sample whith no legumes to 0.27 to 0.49 when the legume proportion ranged from 0.08 to 0.24 in the cow diet. In our study, the proportion of RRR in total PHY ranged from 0.14 to 0.70 (Figure 1) and was lower (*P*<0.05) in group NG (0.20) than in groups Pd+n (0.41), OB (0.45) and BD (0.50). Individual organic and biodynamic farms were rather similar, except one BD farm. RRR share data were non-normally distributed; the Spearman correlation coefficient between RRR share and the share of fresh grass was 0.803. The average RRR share in milk from grazing cows (0.39) was higher than from cows indoors (0.24). In line with this, the SRR/RRR-diastereomers ratio decreased as the amount of grass products in the diet increased, and it was numerically lower (*P* =0.057) in the organic/BD milk compared with the conventional milk samples in this study (Capuano *et al.*, 2014). In another study (Capuano *et al.*, 2013), based on triacylglycerol profile, the milk from cows that had fresh grass in the daily ration could be distinguished from milk from cows that had no fresh grass with sensitivity and specificity values >85%, but authentication of pasture grazing and of organic/biodynamic farming proved difficult during the grazing period.

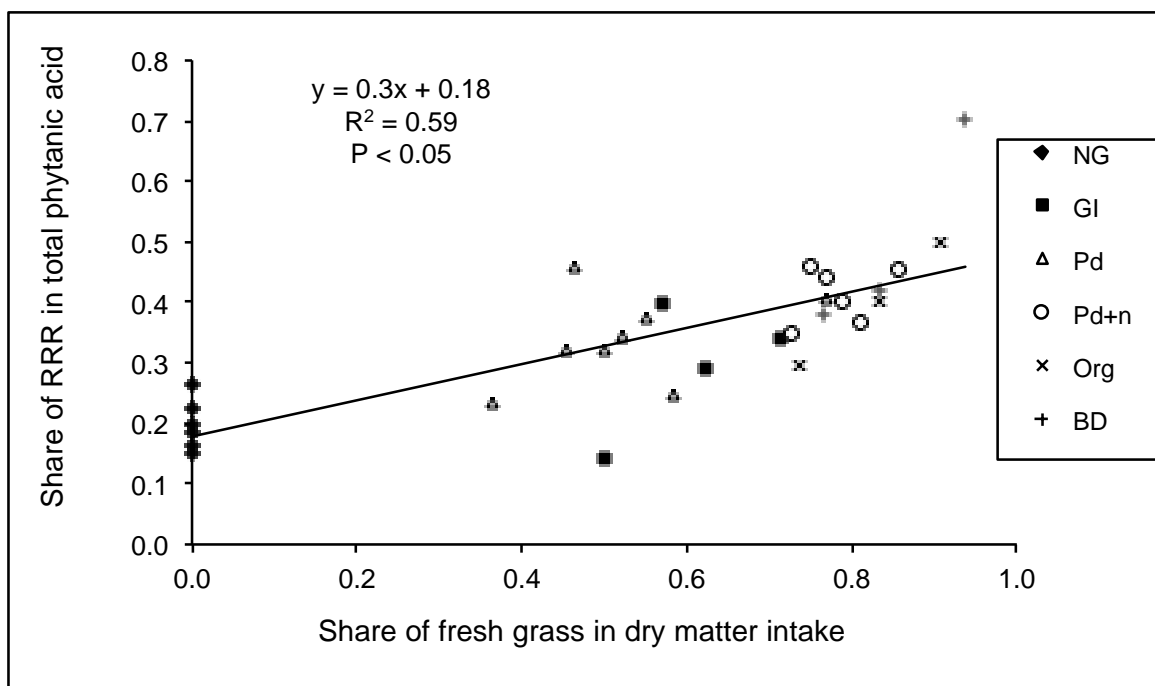Figure 1. Proportion of diasteromer 3R,7R,11R (RRR) in total phytanic acid in individual raw farm milk samples: NG, no fresh grass G; GI, cows indoors with cut fresh grass; Pd, daytime grazing; Pd+n, day + night grazing; OB, Pd+n in an organic (Org) or biodynamic (BD) farming system, in relation to the proportion of fresh grass in the cows' diet.

Conclusion

Concentrations of PHY were not significantly correlated with the amount or proportion of fresh grass nor with the total grass-based forages in the animal diet. PHY cannot be used to authenticate pasture feeding or organic/biodynamic management systems. In contrast, the share of RRR in total PHY showed a good correlation with the proportion of fresh grass in the cow's daily ration. PHY diastereomers might be used for the authentication of fresh grass feeding and/or pasture grazing, along with other biomarkers.

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Potential of fertilized grass clover swards to produce adequate herbage to support dairy cow milk production in high stocking rate grass based systems

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Abstract

White clover (*Trifolium repens* L.; WC) is beneficial in grass swards as it can fix atmospheric nitrogen (N) and make it available for grass growth. This experiment compared herbage and milk production from a perennial ryegrass (*Lolium perenne* L.; PRG) sward receiving 250 kg N/ha/yr (Gr250), a PRG-WC sward receiving 250 kg N/ha/yr (Cl250), and a PRG-WC sward receiving 150 kg N/ha/yr (Cl150) in an intensive grazing system. Forty-one dairy cows were allocated to graze each sward (n = 14). Sward WC content was similar in Cl150 and Cl250 (24.4 and 21.2%, respectively). Cows grazing Cl250 had higher ($P<0.001$) total milk yield than cows grazing Cl150 and Gr250 (6107, 5908 and 5757 kg/yr, respectively). Cows grazing Cl250 and Cl150 had higher ($P<0.001$) total milk solids yield compared to Gr250 (479, 476, 451 kg/yr, respectively). Increased milk production on the clover swards occurred after June. Treatment had no effect on pre-grazing herbage mass. It is concluded that including WC in grass swards can result in an increase in milk production. High N-fertilizer application (250 kg N/ha) did not reduce annual sward WC content compared to Cl150.

Keywords: *Trifolium repens* L., nitrogen, dairy cow and milk production

Introduction

White clover (*Trifolium repens* L.; WC), a legume, is beneficial in grass swards as it can fix atmospheric N and make it available for grass growth. White clover and perennial ryegrass (*Lolium perenne* L.; PRG) have different temperature responses and different seasonal growth patterns (Davies, 1992). As a consequence of low WC growth rates before June/July in temperate regions of the northern hemisphere, the strategic use of nitrogen (N) fertilizer on grass WC swards in spring is commonly practised to increase herbage production relative to swards reliant solely on N fixation. Nitrogen fertilizer application can reduce sward WC content (Harris *et al*., 1996). Research has shown the benefit of WC over PRG for milk production, particularly in the second half of the year (July onwards) (Riberio Filho *et al*., 2003; Egan *et al*., 2013). The objective of the current study was to compare herbage production of and milk production from a grass-only sward receiving 250 kg N/ha with grass WC swards receiving 150 or 250 kg N/ha.

Materials and methods

A farm systems experiment was established at Teagasc, Animal and Grassland Research Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland. Forty-two spring-calving Holstein Friesian dairy cows (33 multiparous and 9 premiparous) were blocked on calving date, pre-experimental milk yield and parity, and randomly allocated to one of the three treatments (n=14), a PRG-only sward receiving 250 kg N/ha/yr (Gr250), a PRG-WC sward receiving 250 kg N/ha/yr (Cl250), and a PRG-WC sward receiving 150 kg N/ha/yr (Cl150), from 17 February to 17 November 2013. All treatments were stocked at 2.74 LU/ha. Fertilizer N application was the same across treatments until May, after which N application to Cl150 was reduced. Herbage was allocated daily to achieve a target post-grazing sward height of 4 cm. Pre-grazing herbage

mass (>4 cm; HM) was determined twice-weekly, using an Etesia mower (Etesia UK Ltd., Warwick, UK). Pre- and post-grazing sward heights were measured daily using a rising plate meter (Jenquip, Feilding, New Zealand). Sward WC content for the clover treatments was quantified once in each paddock prior to each grazing as described by Egan *et al.* (2013). Milk yield was recorded daily and milk composition (fat and protein concentrations) was measured weekly. Milk solids (MS) yield was calculated as the sum of milk fat and protein yields. Data were analysed using PROC MIXED in SAS with terms for treatment, time (week or rotation) and the associated interaction. Fixed terms were treatment and week or rotation, and random terms were cow and paddock.

Results and discussion

There was a treatment × week interaction effect (*P*<0.001) on daily milk yield, MS yield and milk fat content (Figure 1).
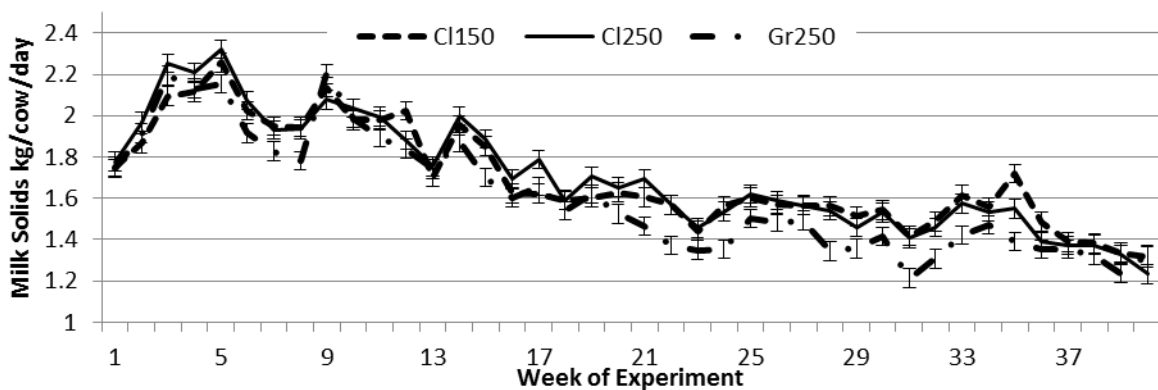
Figure 1. Effect of sward type on daily milk solids production for cows grazing a Grass only receiving 250 kg N/ha (Gr250) and grass clover swards receiving 150 kg N/ha and 250 kg N/ha (Cl150 and Cl250, respectively) for each week of experiment (17 February to 17 November).

All treatments had similar milk yield until experimental week 12 (May) after which Cl250 had higher milk yield compared to the other two treatments until week 16. Milk yield was similar from week 16 to week 19 for all treatments. From week 20 (June) to the end of the experiment, milk yield was greater on Cl250 than on the other treatments; and from week 24 (July) milk yield was higher on Cl150 than GR250 and similar to Cl250. This trend in milk yield was similar to that reported by Riberio Filho *et al.* (2003) and Egan *et al.* (2013). A similar trend was seen for MS yield. The Cl150 treatment had lower MS yield from week 5 to 8; Gr250 had lower MS yield than both clover treatments from week 20 to 25; all treatments had similar MS yield in weeks 26 and 27, and Gr250 had lower MS yield than the two clover treatments from week 28 to 36, after which there was no significant difference between treatments. Daily milk fat content was similar on all treatments at the beginning of the experiment. The Cl150 treatment had higher daily milk fat content compared to the other treatments in weeks 9, 12, 14 and 15 and from week 20 (June) to week 35 (October). Treatment had no effect on daily milk protein. Treatment had an effect on cumulative milk yield (Table 1); Cl150 had lower (*P*<0.05) cumulative milk yield compared to Cl250 (5908 and 6107 kg milk/cow, respectively); there was no significant difference between Cl150 and Gr250 (5908 and 5757 kg milk/cow, respectively); and Cl250 had greater (*P*<0.001) cumulative milk yield than Gr250 (6107 and 5757 kg milk/cow, respectively). There was no significant difference in cumulative MS yield between clover treatments. The clover treatments had greater (*P*<0.001) cumulative MS yield than Gr250 (Table 1). Treatment had no significant effect on pre-grazing HM (Table 1). Sward WC content was affected by the treatment × week interaction (*P*<0.01). Sward WC content was similar between clover treatments from February to July, after which Cl150 had higher WC content for the remainder of the year, which coincided with the reduction in N fertilizer

application to Cl150. Ledgard and Steele (1992) found a similar effect of reduced N fertilizer application on sward WC content. There was no significant difference in the average annual sward WC content between Cl150 and Cl250 (Table 1). Rotation had a significant effect on sward WC content ($P<0.001$) which increased from 0.05 g/kg in February to a peak of 0.43 and 0.33 g/kg in Cl150 and Cl250, respectively, in August.

Table 1. Daily and cumulative milk production and average pre grazing herbage mass on grass-only swards receiving 250 kg N/ha (Gr250) and grass-clover swards receiving 150 kg N/ha and 250 kg N/ha (Cl150 and Cl250, respectively) and average sward clover content on Cl150 and Cl250. ([1]S.E. = Standard Error; [2]TRT = Treatment; [3]Week = Week of Experiment)

	Cl150	Cl250	Gr250	S.E.[1]	TRT	TRT×Week
Milk yield (kg/cow/d)	21.10	21.81	20.56	0.28	<0.001	<0.001
Milk solids (kg/cow/d)	1.70	1.71	1.61	0.02	<0.001	<0.001
Milk fat (g/kg)	4.60	4.41	4.39	0.42	<0.05	<0.001
Milk protein (g/kg)	3.59	3.59	3.58	0.04	NS	NS
Cumulative milk yield (kg/cow)	5908	6107	5757	78.40	<0.001	-
Cumulative milk solids (kg/cow)	476	479	451	5.60	<0.001	-
Pre-grazing herbage mass (kg DM/ha)	1400	1370	1575	90.54	NS	0.07
Clover content (%)	24.4	21.2	-	1.95	NS	<0.01

Conclusions

White clover had a positive effect on milk production regardless of N fertilizer application rate, especially in the second half of lactation. There was no effect of WC inclusion into grass swards on total herbage production. Reducing N fertilizer application in mid-summer increased sward WC content compared to the higher level of N fertilizer. Including WC in grass swards can result in an increase in milk production.

Acknowledgements

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Feeding strategies and feed self-sufficiency of dairy farms in the lowland and mountain area of Western Switzerland

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Abstract

The feeding strategy and the feed self-sufficiency of 34 commercial dairy farms in the western part of Switzerland were investigated throughout the year 2010. Feed ration composition of farms from the lowlands with relatively high proportions of maize were compared to farms from the mountain area with relatively high proportions of herbage in the rations. Dairy feeds were categorized into herbage, whole-plant maize and concentrate. The proportions of dry matter (DM), net energy for lactation (NEL) and metabolizable protein (MP) sourced from herbage, maize and concentrates were calculated. Dairy farms located in the mountain area had higher proportions of herbage DM in the ration than farms located in the lowland (70% vs. 51 %), as well as herbage-sourced NEL (66 % vs. 47%) and herbage-sourced MP (68% vs. 49%). In contrast, lowland farms showed a higher proportion of maize DM in the ration (31% vs. 17 %). The degree of self-sufficiency of the total feed ration of mountain farms was shown to be larger for DM, NEL and especially MP. A general negative relationship between the proportion of maize DM in the ration and the dairy herd MP self-sufficiency could be observed.

Keywords: feed self-sufficiency, feeding strategies, roughage, herbage

Introduction

Grassland-based milk production is of major importance in Switzerland. Traditionally, herbage provides the largest proportion of dairy feeds. Due to climatic and topographic restrictions, maize-based dairy systems are usually limited to the lowland area. Inclusion of higher proportions of maize in the rations consequently decreases the proportion of herbage, which in turn requires the purchase of protein rich concentrates. This feeding strategy increases environmental concerns regarding the importation of soy bean meal and the accumulation of excess nitrogen in dairy farms' environment, decreasing the farms' feed self-sufficiency. On the other hand, feeding strategies relying primarily on the utilization of herbage from grassland are considered environmentally sound and sustainably beneficial. However, with increasing specialization and intensification of agricultural production, grassland-based dairy farms tend to decrease in number in Switzerland. A recently established program "Grassland-based milk and meat production" of the Swiss Federal Office for Agriculture (Schweizerischer Bundesrat 2013), aims to promote the utilization of herbage from grassland and to counteract the tendency of a decreasing feed self-sufficiency of dairy farms. This preliminary field study was conducted to obtain a present overview about the differences in the composition of the feed rations and the degree of feed self-sufficiency of dairy farms in the lowland and mountain area of Western Switzerland.

Material and methods

Thirty-four dairy farms were randomly selected within the cantons of Neuchatel and Waadt (lowland: 22; mountain and hills: 12). The feed rations were investigated throughout the year of 2010. The dry matter (DM) consumption of every dairy herd was modelled on a monthly basis according to herd structure and cow characteristics (parity, live weight, lactation stage,

milk yield; Cutullic *et al.*, 2012). The farmer indicated the proportions or fixed amounts of feeds fed to the dairy cows. As land use area, herd structure and milk production were known (extracted from national databases) the consistency between total consumption and available feeds for the herd, and between milk production and nutrients intake, was checked on-farm during the survey. Dairy feeds were categorized as herbage, maize (whole plant) and concentrate. Herbage and maize were additionally categorized as roughage. The relative proportions of DM, net energy for lactation (NEL) and metabolizable protein (MP) sourced from the different feed components were calculated. To estimate the feed self-sufficiency of the farms, feed components were divided into locally grown (origin < 50 km) or foreign (> 50 km) feeds.

Results and discussion

Lowland and mountain farms had similar area, herd size, total and per cow milk production (Table 1) and DMI per cow and day (Table 2).

Table 1. Characteristics of dairy farms in the lowland (L) and mountain (M) area of western Switzerland (mean ± standard deviation):

	L	M
Farm size (ha)	46 ± 30	41 ± 20
Cows per farm	28 ± 16	22 ± 11
Milk production (t ECM produced per year)	265 ± 162	193 ± 98
Milk production (kg ECM/cow)	25.8 ± 3.9	23.4 ± 4.1

Table 2. Composition of feed rations of dairy farms in the lowland (L) and mountain (M) area of western Switzerland: Average proportions of dry matter (DM), net energy for lactation (NEL) and metabolizable protein (MP) from herbage, maize and concentrates. [1] r.s.e: residual standard error ; Significance[2] ***, *, +, n.s.: $P < 0.001, 0.05, 0.10$, not significant

	L	M	r.s.e[1]	Significance[2]
DM intake (kg) per cow and day:	19.8	19.1	1.4	n.s.
% DM from				
herbage	51	70	12	***
maize	31	17	10	***
roughage	82	86	7	n.s.
concentrates	14	12	5	n.s.
% NEL from				
herbage	47	66	13	***
maize	31	17	10	***
roughage	79	83	8	n.s.
concentrates	18	16	6	n.s.
% MP from				
herbage	49	68	13	***
maize	22	12	7	***
roughage	71	80	1	*
concentrates	26	20	8	+

The composition of the feed ration, however, followed a clear pattern (Table 3): herbage DM proportion was significantly higher for mountain farms (70%) than for lowland farms (51%), resulting in higher proportions of NEL and MP from herbage. As expected, the proportion of maize DM was significantly higher in rations of lowland farms (31%) than of mountain farms (17%). Some of the mountain farms purchased maize cultivated in the lowland area, which explains the occurrence of maize in the feed ration of mountain farms. Inclusion of a higher proportion of maize DM in the rations of lowland farms significantly increased the contribution of maize NEL in the ration and to a lower extent of maize MP, as maize is a protein-poor forage. This was not compensated by an increased contribution of herbage MP, but by the use of dehydrated lucerne and protein-rich concentrates.

Table 3. Degree of self-sufficiency of dairy farms in the lowland (L) and mountain (M) area of Western Switzerland for dry matter (DM), net energy lactation (NEL) and metabolizable protein (MP). [1] r.s.e: residual standard error ; Significance[2] **, *, : $P < 0.01, 0.05$

Degree of self-sufficiency (%)	L	M	r.s.e[1]	Significance[2]
DM	90	94	4	*
NEL	88	93	5	*
MP	80	88	8	**

Conclusions

The feeding strategy of mountain farms, which focuses on a maximum utilization of herbage, results in a high feed self-sufficiency. In contrast, the feeding strategy of lowland farms, which include noticeable amounts of maize, results in a reduced feed self-sufficiency. However, in areas with frequent summer droughts, as in the case of the lowland area of western Switzerland, maize cultivation has also been shown to increase the resilience of a feeding system to drought (Mosimann *et al.*, 2013).

Acknowledgements

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Weather effects and cattle behavioural characteristics

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Abstract

The behavioural response of Hungarian Grey Cattle (HGC) to environmental factors, when managed under rangeland conditions, provides a good representation of the daily life of beef cattle in the absence of human interference. In addition to ethological observations, we collected zoometeorological information that affect cattle behaviour under natural conditions. We found a very complex interaction (neuro-endocrine complex), which utilizes different fields of science including meteorology, ethology, pharmacology, physics and agronomy. This paper's aim is to present our research methods and results about environmental effects, in the context of animal behaviour. The programme has focused on animal behaviour, using GPS technology, but also attempted to collect zoometorological data in alternative ways (ion count, barometric measurements). The applied research methods include spatial data recorded with GPS collars, meteorological information (barometric pressure, wind direction, temperature, weather fronts) and field reports about regular behavioural attributes.

Keywords: behaviour, cattle, GPS, zoometeorology, ion

Introduction

Zoometeorology is an interdisciplinary science, merging the principles of ethology and meteorology. The mutual effects of environmental factors are quite complex (Kovacs, 2010); therefore, they need to be examined in a broad context. Earlier zoometeorological observations (Malechek and Smith, 1975) pointed out that grazing cattle react in a variety of ways to different weather conditions. They have proven that the abiotic environment, animal physiology and behaviour are all correlated.

Materials and methods

The study area is 1191 ha of rangeland. There are two major parts: the North (688 ha) and South (503 ha). We applied the terminology of Czakó *et al*. (1985) and Kilgour (2012) to describe the animal behaviour and organize the behavioural traits into 3 main groups. The feed intake actions (grazing, rumination, drinking), sexual and calf-care actions (copulation, nursing) and social actions (fighting, play, moving). Two types of GPS receivers have been used (Snewi Trekbox, Bluetooth, GT-750 GPS data logger) to describe the animals' spatial position and calculate the speed, daily travel distance and the time spent standing. The loggers recorded animal daily route for 5 days. The positional data have been transformed to digital mapping. Animal behaviour has been observed periodically, every 20 minutes, with recordings of approximately 5 seconds for each. The most typical behaviour pattern was logged. We used binoculars to observe cattle. It was necessary to avoid bothering animals and keep out the flight-zone (average 50 m before cattle change behaviour). The typical behavioural patterns, (grazing, walking, fighting, calf care) were recorded with digital video camera. We also sub-categorized behavioural traits for statistical analyses (see Table 1). Meteorological data were collected from the national meteo-survey database and we have also made local measurements (air pressure, temperature, air ions).

Table 1. Categorization of Hungarian Grey cattle field behaviour.

Behavioural trait	Ethological category	Practical category
Grazing		
Grazing on the move		
Ruminating	Feed intake/Digestive/Nutrient cycle	Feed intake
Drinking		
Excreting		
Walking	Locomotion/Posture	
Lying		
Agonistic/Fight		Social
Grooming	Social behaviour	
Watch		
Mounting/Mounted	Sexual and Parental behaviour	Sexual
Suckling		

Results

There is a significant correlation (r=0.491, *P*<0.01) between weather-front-induced barometric pressure and the length of the herd's daily route. The front-free and cold-front weather systems create high air pressure (P≥1005 hPa at converted altitude) which results in calmer behaviour. The relaxed cattle spend more time feeding. Seeking fresh, nutritious grass is a natural, herbivore behaviour (Gere, 1977); therefore a non-stressed herd's pasture-activity 8 of 10 of time is spent feeding. Results show that warm-weather front – low air pressure (P≤1005 hPa) – causes more stress because of the changing (dropping) air pressure effect on the parasympathetic nervous system (Kovács, 2010) and often combined with high temperature and humidity. The stressed animals gather, spend more time in shade at nearby water-source (river-bank) or continuously roaming on pasture and finally do not complete their average daily route (6-8 km; Haraszti, 1977). We have concluded that a connection exists between stable weather systems and increased feeding activity. The stable weather means high barometric pressure builds up (above 1005 hPa). These conditions occur during cold fronts and the time when no front is present. During such weather, cattle cover more distance to browse for grass (Table 2).

Table 2. Distribution of behavioural traits during different weather system from 2010 to 2013

Front types / Behavioural trait	No Front	Warm front	Cold front
Feed intake	93%	47%	95%
Sexual	2%	10%	0%
Social	5%	43%	5%
Gross count of behavioural traits	100 % (1049 pc)	100 % (368 pc)	100 % (423 pc)

Source: Own calculation;   n=10 – marked cattle

Discussion

The network of physiological processes (serotonin reuptake response, thermoregulation) have created an amazingly flexible bio-system (Phelps, 2005), which eventually drives the cattle

behaviour. We suggest that the incoming weather systems change the air ion concentration, which results with serotonin neuro-transmitter stress. Throughout this process the animal becomes more frustrated (after the short period of serotonin indicated calm period) and spend less time at grazing and with comfort behaviour. The changing weather also has an effect on barometric pressure, which is easier to measure in time-lapse or real-time. Our results show that during calm weather/high pressure periods the herd behaves more balanced than in periods with low pressure/warm-fronts.

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Relationship between the composition of fresh grass-based diets and the excretion of dietary nitrogen from dairy cows

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Abstract

In studies carried out in Aberystwyth, dry and lactating dairy cows were individually fed diets based on fresh grass or fresh grass-clover mixtures that differed in concentrations of crude protein (nitrogen (N) $\times$ 6.25) and water-soluble carbohydrates (WSC). The apparent partitioning of dietary N into milk, faeces, and urine was measured and the relationships between diet composition and N excretion were investigated. Relatively poor correlations between N intake and output of N in milk and faeces were found, but a strong split-line relationship between N intake and excretion of N in urine was detected ($R^2 = 0.79$): up to an intake of 397 g N d^{-1}, mean urine N output was 81 g d^{-1}, and above this the slope of the relationship was 0.786 g g^{-1}. Similarly, a strong negative split-line relationship was found between the whole diet WSC/N ratio and the apparent excretion of dietary N in urine. Below a ratio of 8.94, urine N/feed N = -0.0416 $\times$ diet WSC/N, and above this mean urine N/feed N was 0.199 g g^{-1} ($R^2 = 0.76$). It is concluded that the ratio of diet WSC and N concentrations in fresh grass- and grass-clover-based diets could help predict the losses of N in urine.

Keywords: grass, water-soluble carbohydrates, dairy cows, nitrogen excretion

Introduction

Significant but variable quantities of nitrogen (N) are excreted in the urine of dairy cows, where it contributes to pollutants such as nitrous oxide, ammonia and nitrates (McGechan and Topp, 2003). Leached losses of N from grazing pastures primarily originate from urine patches, and Li *et al*. (2012) suggest better knowledge of urinary-N deposition in urine would enable improved prediction of leached N losses from soil. While a key factor determining the excretion of N from dairy cows is N intake (Kebreab *et al*., 2001), the apparent partitioning of dietary N between productive purposes (i.e. milk protein) and excretion is influenced by diet composition and the effective use of N by rumen microbes. Increasing rates of N fertilization increases grass N concentration, but this does not necessarily lead to increased duodenal N flow in animals that eat the grass (Peyraud and Delaby, 2006). Surplus diet N is lost in urine following absorption as ammonia from the rumen when the rumen population has insufficient energy sources to capture it. Increased concentrations of grass water-soluble carbohydrates (WSC) have been shown to reduce the proportion of dietary N that is excreted in urine (Miller *et al*., 1999; Miller *et al*., 2001; Moorby *et al*., 2006), but at some point ammonia absorbed from the rumen will be minimal and further increasing grass WSC concentrations will have no additional benefit. This work investigated the relationships between the nutritional characteristics of fresh ryegrass-based diets and the excretion of N from dairy cows.

Materials and methods

A number of experiments have been carried out in Aberystwyth over the last 15 or so years in which individual dairy cows were offered zero-grazed diets based on *ad libitum* access to fresh grass or mixtures of fresh grass and white clover (Miller *et al*., 1999; Miller *et al*., 2001; Moorby *et al*., 2006; and J.M. Moorby, unpublished data). Diets were designed to create differences in the WSC and N concentrations of forage, and were supplemented with relatively small amounts (3-4 kg d^{-1} per cow) of concentrate feed. Data from 55 whole-body N partitioning measurements carried out to determine the apparent partitioning of feed N into milk, urine and faeces were

available. Briefly, intakes of fresh feeds were recorded and total collections of faeces and urine were carried out for 6 days. Concentrations of N in feeds, milk, faeces and urine were determined to allow calculation of daily inputs and outputs of N (g d^{-1}), and apparent N partitioning was calculated by relative differences in intake and outputs (g output g^{-1} intake). Individual cow data were analysed by correlation analysis and split-line ('broken stick') regression using Genstat (15th Edition, VSN International Ltd, UK).

Results and discussion

Diet dry matter (DM) intakes and milk yields varied widely among the experiments (Table 1), and were largely dependent on the stage of lactation of the dairy cows used, which ranged from early to late lactation. Whole-diet concentrations of N and WSC varied by a factor of 2 across the experiments (whole diet crude protein ranged from 113 to 228 g kg^{-1} DM). Apparent outputs of diet N in milk and urine both varied by a factor of 3.6.

There was a relatively poor correlation between diet N intake and outputs of N in milk ($R^2 = 0.29$) and faeces ($R^2 = 0.45$). However, there was a significant ($P < 0.001$) split-line relationship between diet N intake and urine N output (adjusted $R^2 = 0.79$; Figure 1). There appeared to be little relationship between N intake and urine N output up to an intake of 397 (s.e. 15.6) g N d^{-1} with mean urine N output at 81 (s.e. 6.9) g d^{-1}, but at higher N intakes the relationship was urine N output = 0.786 (s.e. 0.0775) × diet N intake (g d^{-1}). A strong relationship between N intake and N excretion is well know (Kebreab *et al*., 2001), and in this case, above dietary crude protein intakes of approximately 2.5 kg d^{-1}, more than 78% of dietary N was apparently excreted in urine.

Table 1. Mean and standard deviation (SD) and range of selected parameters of individual dairy cow measurments (n = 55) in which the apparent partitioning of feed N was measured

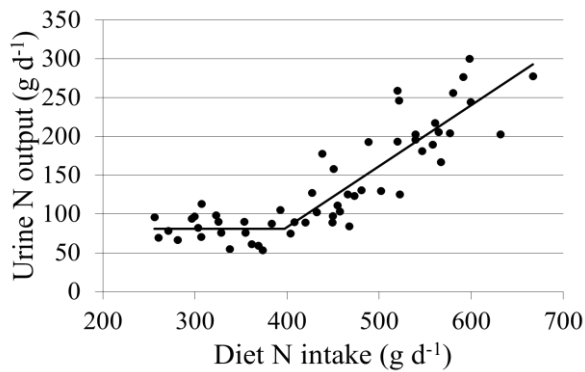|	Mean	SD	Min	Max
Dry matter intake (kg d^{-1})	17.7	1.8	12.2	20.5
Diet N (g kg^{-1} DM)	24.9	5.2	18.0	36.5
Diet WSC (g kg^{-1} DM)	160	38	103	217
WSC intake (g d^{-1})	2853	855	1297	4381
N intake (g d^{-1})	443	108	256	667
Diet WSC/N (g g^{-1})	6.73	2.21	3.21	10.86
Milk yield (kg d^{-1})	24.0	8.3	9.6	40.0
Milk N out (g d^{-1})	124	37	63	200
Urine N out (g d^{-1})	138	68	54	300
Milk N out/Feed N in (g g^{-1})	0.29	0.07	0.12	0.43
Urine N out/Feed N in (g g^{-1})	0.30	0.09	0.14	0.50

Figure 1. Relationship between diet N intake and urine N output in individual cows fed diets based on fresh grass
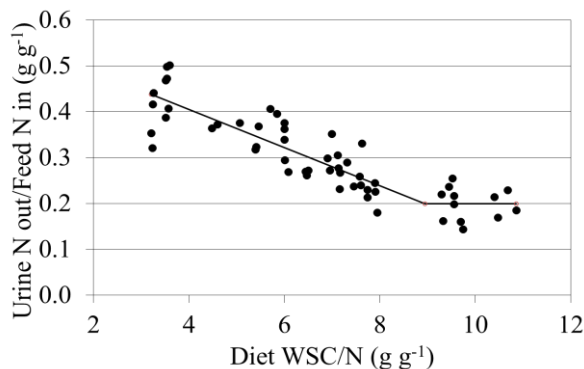

Figure 2. Relationship between whole diet ratio of WSC to N and apparent excretion of feed N in urine from dairy cows (n = 55).

Dietary intake of WSC was poorly correlated with daily outputs of N in milk ($R^2 = 0.27$), urine ($R^2 = 0.11$) and faeces ($R^2 = 0.01$). However, the ratio of WSC to N concentrations in the whole diet (g g^{-1}) was negatively related to the apparent excretion of feed N in urine in a significant ($P < 0.001$) linear split-line relationship of urine N/feed N = -0.0416 (s.e. 0.0041) × diet WSC/N up to a diet WSC/N ratio of 8.94 (s.e. 0.457) g g^{-1}. After the breakpoint, the apparent excretion of dietary N in urine was 0.199 (s.e. 0.0125) g g^{-1}. The adjusted R^2 (0.77) of this relationship was marginally better than the coefficient of determination of a standard linear regression ($R^2 = 0.76$). A negative relationship between diet WSC/N and apparent urinary excretion of dietary N has been noted before (Edwards *et al.*, 2007), using some of the current data. The current study builds on previous (and different) data and indicates that the negative relationship holds when including clovers in the diet of the cows. The split-line relationship also suggests that beyond a ratio of WSC to N concentrations in ryegrass-based diets of approximately 9 there is little additional benefit to increasing the concentration of WSC in terms of reducing the apparent excretion of dietary N in urine. Further work is required to investigate whether the relationship is maintained at higher ratios of WSC to N because the current dataset is relatively small at this end of the range.

Conclusion

In dairy cow diets based on grazing, grass WSC concentrations play an important role in helping rumen microbes capture grass N. The current results suggest that at typical grass crude protein concentrations between about 160 and 200 g kg^{-1} DM (about 26 to 32 g N kg^{-1} DM), mean WSC concentrations of between about 230 and 290 g kg^{-1} DM would minimize rates of urine N excretion in grazing dairy cows. As a target for ryegrass breeding programmes, this should be relatively easily achieved.

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Theme 5 'MultiSward'

Theme 5 invited paper

Multi-species swards and multi scale strategies for multifunctional grassland-base ruminant production systems: An overview of the FP7-MultiSward project

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Abstract

MultiSward aimed to conceive, evaluate and promote sustainable ruminant production systems based on the use of grasslands with a high level of multi-functionality by the concerted use of diverse multi-species swards, diverse plant communities at farm and landscape levels, and diverse production systems. It was demonstrated that the use of sown multi-species swards combining legumes and grasses as well as shallow and deep-rooting species are as productive as fertilized grasses; they allow high levels of animal performance and these results are not conflicting with the delivery of a range of services. For permanent grassland, differentiated grassland management and climate variables influencing functional diversity criteria linked to ecosystem services were identified. Several innovations in grazing management were proposed to enhance the benefits of grassland-based systems. Different operational indicators sets easily implemented at farm and at landscape levels were developed and demonstrate the contribution of grassland-based systems and effects of management on the provision of food and environmental services including the maintenance of farmland biodiversity. Modelling of several policy options and price hypothesis showed that extensive grassland abandonment may be prevented by area payment supporting grassland use, whereas lower Common Agricultural Policy support payments and higher commodity prices may cause less control of land use. In conclusion, MultiSward provided a detailed EU-wide and multifunctional-orientated overview of grassland-based ruminant production systems thus opening the opportunity to define, on a new basis, the contribution of ruminant production on grassland to economic returns for farmers, biodiversity conservation and provision of ecosystem services. This will lead to a better consideration and acceptance of these ruminant production systems by EU citizens and farmers, and will contribute to secure optimal European grassland acreage.

Keywords: multi-species swards, grazing, ruminants, competitiveness, public goods, public policy

Introduction

Grasslands are the main survival resource for about one billion people worldwide. In industrialised Europe grasslands cover some 39% of the agricultural area and form the basis of a strong ruminant livestock sector (Huyghe *et al.,* 2014). Grasslands are not only a relatively cheap source of feed for ruminants, but are also increasingly being recognised for their contribution to the conservation of biodiversity, to the regulation of physical and chemical fluxes in ecosystems, to the mitigation of pollution and to the production of landscape amenity (De Vliegher *et al*., 2014). The relative importance of the multiple functions provided to society by grasslands varies depending on regional contexts and grassland type and are strongly

influenced by the type of management: extensive, species-rich and nutrient-poor systems result in less 'provisioning' services but are more effective at providing other types of services than intensive grassland-based systems. These functions are well recognized and appreciated by relevant stakeholders as shown by an on-line questionnaire developed during the MultiSward project (Van den Pol-van Dasselaar *et al*., 2014).

Grassland acreage has been significantly reduced in Europe during the last thirty years (by approximately 15 M ha, i.e. 30%) in favour of the production of fodder maize and other annual crops ; even marginal grasslands tend to be abandoned, particularly in mountainous and Mediterranean areas. This was the consequence of farming systems intensification, specialization of production, decrease in cattle population, price support, premium systems and farm size growth (Huyghe et al., 2014) whereas in the same time the production of public goods in not (or poorly) remunerated by the market or the public policy. At the same time the simplicity of managing grass monocultures and the low price of mineral nitrogen have inhibited the use of legumes for forage production. EU Common Agricultural Policy (CAP 1992 with the premium to cereals) has helped shape the current decrease of Europe's grassland areas (Peyraud *et al*., 2012). However, new opportunities recently appeared for grassland based systems. Rising global demand for meat and milk, European concerns about environment preservation and food quality and safety favour the increasing role of sustainable grassland-based ruminant systems in the future. Climate change mitigation policies could support the conversion of arable land to grassland for carbon sequestration. After the CAP reform in 2003 cross-compliance rule on the protection of permanent grassland area, rural development expenditures and less Favored Areas payments are *a priori* more favourable tools for the maintenance of the permanent grassland. In the same time, farming practices can have both positive and negative externalities and too little is known about how well the different grassland management systems and their localisations perform in delivering ecosystem services. This clearly stresses the necessity of developing comprehensive studies of the influence of different grassland management strategies in different local conditions on the positive and negative externalities of the production from field to landscape level.

In this context, the main ambition of MultiSward is to conceive, evaluate and promote sustainable ruminant production systems based on the use of grasslands with a high level of multifunctionality in order to optimize economic efficiency, the provision of environmental goods and biodiversity preservation. MultiSward considered multi-species swards (MSS) because in fertile agrosystems MSS can reduce energy consumption by replacing highly energy demanding nitrogen (N) fertilizer by natural nitrogen fixation, whilst maintaining biomass production (Lüscher *et al*., 2014) and in relatively species-rich and nutrient-poor systems, the provision of ecosystems services is generally enhanced by species diversity. To achieve this ambition, the main objectives were to (i) identify and analyse the effects of socio-economic and policy scenarios on the future of grassland acreage and identify under which policy conditions sustainable grassland systems will become a viable option for farmers compared to crop-based systems; (ii) assess and optimize the performance of MSS to enhance the competitiveness of grassland based production systems and the provision of regulating and supporting services; (iii) design and evaluate innovations in grazing and animal management (including animal genetics) to assess the best way of combining high production efficiency, competitiveness and provision of services. MultiSward covered five main biogeographical regions (Atlantic, Continental, Alpine, Mediterranean, Boreal) and considered high inputs and low inputs systems, productive grassland and nutrient poor systems encountered across Europe thus covering a large array of ecosystem services.

State of grassland and grassland based systems in Europe

Evolution of grassland acreage in Europe

MultiSward has provided a detailed view of grassland acreage and utilisation in Europe. Permanent grasslands (PG) cover over 57 million ha in the EU-27 (2007), temporary grasslands (TG) about 10 million ha. Together, they occupy about 39% of the European Utilized Agricultural Area (UAA). Grasslands still cover the largest agricultural area. Permanent grassland area is very important in Ireland (75% UAA), UK (58% UAA), Slovenia (58% UAA) and Austria (55% UAA) (Huyghe et al., 2014). In terms of number of hectares the United Kingdom (11 million ha), France (9.8 million ha), Germany (4.8 million ha), Italy (4.5 million ha) and Romania (4.5 million ha) represent 62% of the total permanent grassland area in EU-27. These grasslands are the basis of the feeding of about 78 million Livestock Units (LU) of grazing livestock. They are managed by about 5.4 million holders, i.e. about 40% of all European farm managers. Among these farms managing permanent grasslands, 41% have an Economic Size Unit (ESU) lower than one (very small farm). The European grassland area has been significantly reduced during the last 30 years there were large differences in evolution trends between countries. Losses were very important in Belgium, France, Germany, Italy and The Netherlands while surfaces remained almost stable in Luxemburg, United Kingdom and Ireland. In the EU-6, these losses are estimated at about 30% and 7 million ha between 1967 and 2007 (Eurostat, 2009).

Behind these mean figures, estimates vary according to the sources of information (Eurostat, FAOSTAT and national databases) and even within databases over time because the definition of grassland is variable according to the source, terms are often used in an imprecise and misleading way (for example terms like 'meadows' and 'pastures'), the term 'rough grazing' does not represent all species-rich grassland types, Temporary grasslands are recorded as 'Leguminous plants' and 'Temporary grass' which induces doubt in the classification of grass-legume mixtures and changes in survey methods occurred over time in some countries (Greece, Italy, Portugal). This clearly obscures the vision of various stakeholders and does not allow taking into account all the diversity of grasslands by the Common Agricultural Policy (CAP). A better definition and classification of grassland terms should help to optimize the supports for grassland according to the services that they can provide and to secure grassland acreage in Europe with well targeted premiums. A comprehensive classification of grassland types was provided by an EGF / MultiSward group of experts coordinated by A Peeters. The proposal is described elsewhere (Peeters *et al*., 2014). It is a compromise between the practical aspects related to data collection and the level of precision that is necessary to reach the objectives described above and consist in (i) the classification of temporary grasslands into three categories: pure legume sowings, pure grass sowings and grass-legume mixtures; (ii) the classification of permanent grasslands into three categories: agriculturally-improved, natural and semi-natural, no longer used for production; and (iii) the introduction of a new category for grazed fallow land.

Services provided by grassland area and grassland based production systems in various European regions

To evaluate the impacts of ruminant production systems on the quality and the use of natural resources (air, water, soil, energy, biodiversity) and to assess a large range of ecosystem goods and services provided by grasslands and grassland-based systems an indicator system was developed and implemented. This system (MultiSward Indicator System - MIS), focuses primarily on provisioning services (production of food and feed), regulating services (atmosphere regulation with GHG and NH3 emissions; water quality protection: gross nitrogen and phosphorus balances, pesticide use), supporting services (biological N fixation), cultural services (landscape quality). The MIS is inspired by recent and effective systems, and

particularly by the 28 agri-environmental indicators of the European Commission (2006) calculated at country level. The scope of MIS is more restricted than the agri-environmental indicator system of the European Union but it is as much as possible compatible with this system. The MIS structure is based on the Driving force — Pressure — State — Impact — Response (DPSIR) framework of the European Environment Agency (EEA, 1999). Another characteristic of the MIS is that it includes two lists; one is calculated per farm type for a selection of regions, the second is calculated per region for the same selection (all farm types merged), while the EC indicator list is calculated at country level (all regions and farm types merged). The lists adapted by MIS to farm types and to regions levels include respectively 23 and 45 indicators. This conforms with EUROSTAT (2013) requirements that consider that regional data are of particular importance for agriculture and especially for the new risk indicators. MIS was applied in 16 NUTS 2 regions (Figure 1) corresponding to a large range of pedo-climatic, geographic and social conditions in Europe and to a large range of farming systems. Regions were chosen according to the typology of livestock regions described by Pflimlin *et al.* (2005) and EEA (2001).
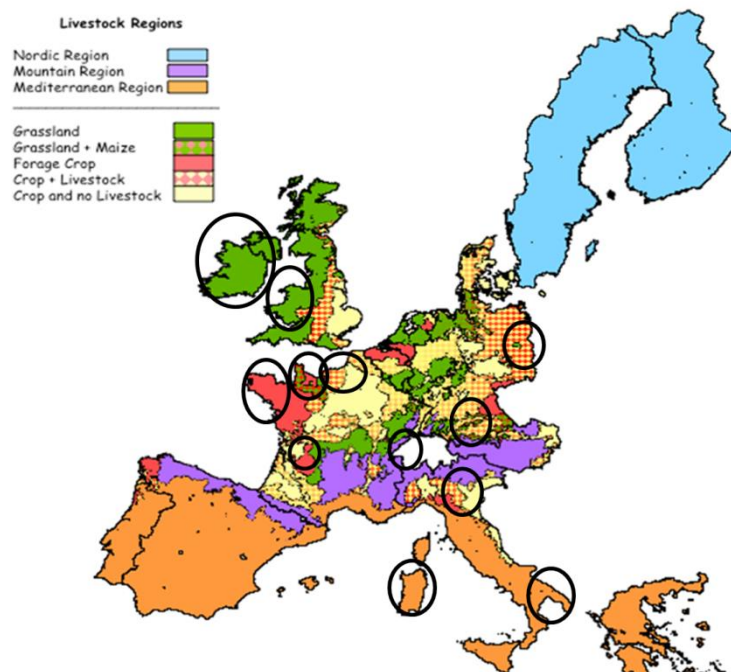
Figure 1. Livestock regions of Europe with the indication (circles) of regions studied. Grassland regions of the lowland (permanent grassland (PG) > 40% of agricultural area (AA) and less than 10% maize forage (MF) in main forage area (MFA)); Grassland and maize regions (PG/AA > 40% and MF/MFA < 10%); forage crop regions (PG/AA < 40% and MFA/AA > 50%); arable land and livestock (PG/AA < 40% and 20 < MAF/AA <50%); arable land and no livestock (PG/AA < 40% and MFA/AA < 20%). NUTS2 regions were Centre France (FR24), Brittany (FR52), Lower and Upper Normandy (FR25, FR23), West and East Wales (UKL1, UKL2), Midland-western and Southern-Eastern Ireland (IE01, IE02), Niederbayem (DE22), Zentralschweiz (CH06), Wielkopolskie voivodship (PL41), Trentino (ITD1), Alto-Adige (ITD2), Puglia (ITF4), Sardinia (ITG2).

The utilisation of the MIS highlighted some contrasting evolutions between European regions and provided some original information on economic and environmental performances both at farm and regional levels. Although the permanent grassland area is still decreasing at the scale of Europe (especially in North West Europe), permanent grassland areas increase (+8% ITG2) or are relatively stable in 'Permanent Grassland' regions. Beef cattle farms are the best guarantor of these surfaces since dairy farms tend to use more green maize, when possible, temporary grasslands and even cereals in the diet of their productive animals. Permanent grassland areas decrease, sometimes quickly, in 'Permanent Grassland & Maize' and 'Arable Land & Livestock' regions (- 5 to -7% between 2000 and 2010 for FR252-253, FR23). That

shows that green maize exerts a powerful pressure on permanent grassland when cropping of this crop is possible and understood by farmers. In 'Forage crops regions', it seems very easy for farmers to convert temporary grasslands into green maize and other annual crops (cereals). Annual yields of agriculturally improved permanent grasslands were estimated (on the basis of expert knowledge) as ranging between 5 and 10 t DM ha^{-1}. Annual yields of temporary grasslands were assessed at about 9 to 16 t DM ha^{-1}. In Mediterranean regions both grassland type yields are lower. The high yields of temporary grassland swards can be partly explained by a good proportion of legumes.

The MIS also provides information on the environmental performances of farming systems. At regional level, ammonia emissions (from 9 to 45 kg N-NH3ha^{-1}) are best correlated with monogastric stocking rate, GHG emissions (0.8 to 4.7 t CO2 eq ha^{-1}) with grazing livestock stocking rate (highest value for IE01, IE02, FR52, DE22 and DE21) and gross nitrogen balance (35 to 140 kg N ha^{-1}) with total livestock stocking rate. Biological nitrogen fixation is low everywhere in permanent grasslands (< 25 kg N ha^{-1}) showing that grassland productivity relies still on nitrogen fertilisation although some regions such as Wales (UKL2 = 70 kg ha^{-1}) and Central Switzerland (CH06 = 52 kg ha^{-1}) are however able to devote a much higher proportion of N to legumes in grassland swards. Biological nitrogen fixation is, however, much higher in temporary grassland (73 to 225 kg N ha^{-1}) with the noticeable exception of Ireland (IE1 and IE2 = 2 kg ha^{-1}). Incorporation of lucerne, red clover and other nitrogen fixing legumes is indeed common in temporary grassland mixtures. Average soil organic carbon (SOC) density in grassland soils is considerable, about twice the value of arable land in our sample and reaches 300 t CO$_2$ eq. ha^{-1}. Regarding global warming potential, gross nitrogen surplus and ammonia emissions, livestock farms are in average 2 to 5 times more polluting per surface unit than arable farms. However, this negative impact on the environment is lower than for 'Specialist granivores' that emit 10 to 100 times per surface unit more than arable farms. Moreover, grassland-based systems store carbon in soil organic matter which compensates GHG emissions and have also an important capacity to absorb nitrogen surplus in their SOC.

Concerning biodiversity conservation, it is noticeable that specialised grazing livestock regions considered all together maintain and establish more landscape elements than arable land regions. The proportion of High Nature Value (HNV) farmland in the agricultural area is relatively high on average in Grassland regions but reflects mainly national policies. It. is high in Wales (46% AA) but low in areas where this proportion could be very high, like in the Italian Alps and Mediterranean areas as well as in Poland (8% AA). The number of 'Natura 2000' protected habitats in grassland is logically high in the Alps and in Poland (*i.e*, 7 to 10) but surprisingly low in the Mediterranean areas of Italy. Even in grassland regions, the trend of the populations of farmland birds is sharply decreasing although exceptions, like in Wales, show that an adequate management of landscape and favourable agri-environmental policies can revert the trend. 'Specialists small ruminants' (sheep, goats and other grazing livestock) are the best managers of biodiversity. They are the main managers of rough grazing areas and are characterized by a high level of agricultural biodiversity (livestock species diversity). They also maintain landscape elements very well. 'Specialist dairying' are much less efficient in terms of biodiversity conservation but they also maintain landscape elements that are important for wildlife. 'Specialists monogastrics', 'Specialists vineyards' and 'Specialists arable crops' are the worst managers of ecological infrastructures despite the fact that, in some regions, 'Specialists arable crops' can use and maintain rough grazing areas, probably with sheep flocks that constitute a marginal part of their income

Identification and analysis of the effects of socio-economic and policy scenarios on the future of grassland acreage

After the radical reform of the CAP in 2003 the agri-environment measures have contributed to reduce the loss of permanent grassland (Peyraud *et al*., 2012), but much of the more intensive grassland areas will have remained outside of these schemes and may be used for cropping when commodity prices make this more profitable. MultiSward has identified some external drivers that can support grassland-based systems or at the opposite threaten them by assessing the likely effects of the revised pillar 1 CAP policies and how modified payment schedules could influence farmer's decision to use land for growing grass or other crops in the case of 3 countries (Switzerland, Germany, Wales) (Hecht *et al*., 2014) using FARMIS model. Specifically, the analyses intended to quantify from current policy frameworks (baseline) alternative specific policies scenarios which could support grasslands and grassland-based systems under different market conditions or. The rationale for the choice of Germany, Wales and Switzerland is that the three countries differ considering the role and importance of grassland faming and the existing agricultural policies and direct payment system considered in the base year. The FARMIS agri-sector model (Offermann *et al*., 2005; Sanders *et al*., 2008) was used to model likely farmer behaviour with regard to grassland use and consequences on the basis of socio-economic outputs and environmental outputs (e.g. GHG emission, biodiversity, nutrient losses).

Strong variations in input and output prices (50%) have a very significant effect on grassland area, the intensity of management of grassland systems and emissions to the environment, and are a key driver for the profitability of grassland systems. The high output price scenario results in farming intensification and overall production increases (milk by +29% in Germany, +29% in Wales, +51% in Switzerland; beef output by +17% in Germany, +43% in Switzerland; and sheep by +13% in Wales) and more fodder is grown on arable land; temporary grassland increases while extensive grassland use is strongly decreasing (-42% in Germany - especially in the intensive dairy production regions of Schleswig-Holstein, Lower Saxony and Bavaria, -55% in Wales). This scenario also leads to environmental problems. Increased input prices (fertilizers, energy, concentrates) in the baseline scenario led to a significant decrease in Farm Net Value Added in real terms according to countries and farm types. Dairy farms can maintain their income level, as farm and productivity growth and an increase in milk production can compensate for rising input costs while, in contrast, beef farms see their income declines which raises serious questions with respect to their economic sustainability in the long term.

The considered policy scenarios had two common themes among countries (i) PREM1: greater payments to permanent grassland from 100 to 250€ ha^{-1} according to the country (in Germany with re-allocation of 15% budgets from the first pillar, in Wales and Switzerland with a "top up" payment); and ii) PREM2: greater payments to extensive grassland (*i.e.* +250€ ha^{-1}), with reduced payments to arable land (-40€ ha^{-1} on the mean).

The results for the area payment scenarios with and without transmission of payments from arable land illustrate the potential of such payments in maintaining grassland acreage and in further harmonising income levels between farm types, but this may not work for all areas. Whilst this appeared to work successfully in Germany and Switzerland, in Wales and other grassland-dominated areas, the potential budget transfer from arable areas is low, and therefore capacity for increased grassland payments is reduced. These payments have limited impacts on emissions except in Switzerland where support to permanent grasslands reduces N-eutrophication through a reduction of arable land and temporary grassland. Regarding the scenarios in extensive grassland and on differentiated supports, there are some contrasting results. In Germany, the support of extensive grasslands is very positive for the extensive grassland area and for farmer's income. In Switzerland, the best option is the differentiated support to grassland types (semi-natural > agriculturally improved permanent > temporary

grasslands). It has a very positive impact on the semi-natural grassland area, on farm income and positive but limited impact on N-eutrophication.

Table 1. Impact of different market and policy scenarios on land use, production and income at the sector level in Switzerland (CH), Germany (GE) and Wales (W). Data expressed in % change to baseline scenario

	IP+50			OP+50			PREM1			PREM2		
	CH	GE	W	CH	GE	W	CH	GE	W	CH	GE	W
Arable land (ha)	-2	-2	-3	3	0	0	-18	0	0	-40	0	-4
Permanent Grassland (ha)	0	-2	-7	0	0	2	0	0	2	7	0	-1
Intensive grassland (ha)	0	-10	-14	-2	16	25	21	0	1	46	-30	0
Extensive grassland	4	18	5	0	-42	-55	2	1	8	23	80	-5
Number of dairy cows	-4	1	-9	51	29	29	0	0	0	14	-1	0
others ruminants	-12	-19	-22	37	22	12	0	0	2	-4	-1	-2
Farm income (€ AWU^{-1})	-10	-24	-	105	81	629	10	0	220	6	0	11
N balance (kg ha^{-1})	-2	-7	-	8	7	-	-14	0	-	-22	-1	-
CH$_4$ (kg eqCO$_2$ ha^{-1})	-2	-	-10	83	-	20	0	-	1	13	-	-1

"IP_+50"=Increase in input prices; "OP_+50"=Increase in output prices; "PREM1"=Increase in grassland payments; "PREM2"=Increase in support for extensively managed grasslands (+ transferred from arable land)

Assessment and optimisation of the performances of MSS

While the agronomic benefits of grass-legume mixtures over grass monocultures have been recognized for a long time, the simplicity of managing grass monocultures and the low price of nitrogen (N) fertilizer have in the past inhibited the use of MSS for forage production in many European countries (Peyraud *et al.*, 2009). Field experiments have shown both higher and lower herbage yields from MSS than from monocultures, depending upon species composition, weather conditions, and management (Sanderson *et al.*, 2004, 2005). In MSS, the 'diversity effect' has been defined as the excess of mixture performance over that expected from component species' monoculture performances (Loreau, 1998). A highly species-rich system (> 30 species) may not necessarily meet farmers' primary objective of producing high yields in productive and stable environments (Sanderson *et al.*, 2004). It would clearly be useful in practical terms if the diversity effect did not rely on highly species-rich communities, but instead could be obtained with a mixture of a few species, well adapted to the appropriate environmental conditions.

Plant diversity increases herbage production

There has been a clear need for experimentation under realistic agronomic conditions to provide definitive evidence of the benefits of sward diversity to farmers in terms of primary (plant) and secondary (animal) production. A previous pan-European study (Kirwan *et al.*, 2007) suggested that a major diversity benefit for yield in agricultural grasslands in temperate regions can be achieved with as few as four well-adapted plant species and these benefits occurred over a wide range of climatic conditions and nitrogen fertilization levels (Lüscher *et al.*, 2008). In the MultiSward 'Common Experiment' (CE), agreed protocols were managed at seven sites and five countries during three years it was hypothesised that using grass/legume mixtures comprising a small number of strategically chosen species for forage production would be a viable option for achieving sustainable intensification of grassland-based agricultural production. Two grasses and two legumes were used. The plots were established based on 4 different 'functional groups' as follows (Collins *et al.*, 2014): Nf = non-N fixing, Fi = N-fixing; Sr = shallow rooting; Dr = deep rooting. The latter contrast was included because enhanced nutrient uptake from a larger soil pool has been suggested to contribute to over yielding in mixtures (de Kroon, 2007; de Kroon *et al.*, 2012). Mixtures of the following species were established: perennial ryegrass (Nf-Sr)*, Festuca arundinacea* (Nf-Dr), white clover (Fi-Sr) and

red clover (Fi-Dr). In one treatment the second grass was replaced with chicory (*Cichorium intybus*) at some of the experimental sites (non-N fixing) or chicory was added as a fifth treatment. Chicory is a deep-rooted forb with a high nutritive value and is well-adapted to dry summers (Barry, 1998). The quantity of applied N was related to the expected biomass productivity (low = 12, high = 25 kg N t^{-1} expected DM).

Results investigating primary production using cut swards (Collins *et al*., 2014; Figure 2) clearly showed that there was no detriment to DM yield in legume-based MSS compared to grass monocultures receiving high inputs of external nitrogen fertilizer. Indeed, in some instances MSS were more productive than the latter thus confirming previous results. Increased use of MSS therefore potentially represents a substantial economic and environmental saving when the various costs associated with the use of nitrogen fertilizer are considered. The trends shown by the results at some (AU-IBERS), but not all (Agroscope) sites indicating an increase in biomass production from one to two and from two to four species in the sward, as well as a predominant role played by the legumes, were in agreement with the results of the previous studies (Kirwan *et al*., 2007). However, over all time scales significant changes in the contribution of sown species to total biomass in mixtures occurred over time in MSS. This illustrates the complexity of species dynamics in MSS.
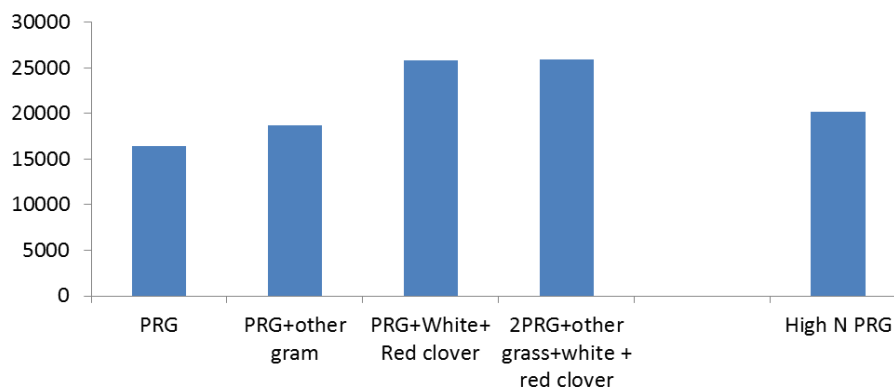
Figure 2: Mean cumulative DM yield (sown + unsown species, kg ha^{-1}) over 3 years in Aberyswyth (12 harvests) and Tänikon (18 harvests) in cut plots (PRG:perennial ryegrass).

A novel aspect of the CE was the defoliation management ('grazing vs. cutting') comparison between biomass production in MSS and grass monocultures carried out at a subset of three sites (sheep in AU-IBERS; beef cattle in Agroscope-Tänikon; dairy cows in INRA-Rennes). There were no clear effects on sward yield of cutting versus grazing and no interactions between defoliation management and sward type with cattle and dairy cows. Grazing with sheep had no effect on grass-based swards but reduced sward yield in legume-based swards. Sheep grazing appeared to have a direct and detrimental effect on the legume component of MSS and this result support the hypothesis that selection of different species or sward types by grazing sheep can have a large effect on the yield, stability and composition of MSS, but that the identity of the grazing animal is the key determinant. Yarrow and Penning (1994) have previously found that the proportion of white clover in mixtures was lower when they were grazed by sheep than if the swards were harvested by cutting.

Plant diversity increases animal performances and production of animal product on a per hectare basis

Research on diversity-productivity relationships in sown grassland has generally focused on primary production, rather than performance of grazing animals. Interactions between different forage elements of the diet can occur and modify forage intake, metabolic processes in the rumen itself and emissions to the environment. MultiSward entails investigations of animal responses to complex swards to better understand the effects of interactions that can occur

between plants on digestion and intake. Experiments were conducted as part of the CE with ruminants fed indoors *ad libitum* to estimate the voluntary dry matter intake and in grazing situation. One experiment also considers permanent grassland.

Indoors experiments with *ad libitum* feeding showed that voluntary DM intake was positively related to diet complexity in sheep, cattle and dairy cows. Three indoor feeding experiments were conducted with sheep fed *ad libitum* with mixtures of forages in controlled proportions (in %, 100:0; 75:25; 50:50; 25:75; 0:100). These experiments showed a positive effect on voluntary intake of dry matter between (i) silage of cocksfoot and red clover when increasing the proportion of red clover in the mixture, (ii) fresh rye grass and chicory when increasing the proportion of chicory and (iii) fresh ryegrass and white clover when increasing the proportion of white clover (Niderkorn *et al*., 2014). Synergy between some species in binary mixtures were observed (Experiments i and iii), optimal for the proportion 50:50, the percentage differences between the values measured for the plant combinations and the balanced median values from pure forages were +9.5% and +5.6% respectively. A cattle experiment (Morel *et al*., 2014) also suggested a greater voluntary intake for a mixture of four forages (ryegrass, chicory, white clover and red clover) compared to pure stand ryegrass (9.2 vs 8.8 kg DM day^{-1} respectively). These results agreed with previous data showing that a mixture of several forages could stimulate the motivation to eat and level of intake (Niderkorn and Baumont, 2009). Moreover, voluntary DM intake of legumes measured on sheep at maintenance is 10 to 15% greater than that of grasses of similar digestibility, and this is true whether legume forages are fed as silage, hay or fresh (INRA, 1989). Dewhurst *et al* (2003) also reported than silage DM intake is increased by 2 to 3 kg when cows are fed with red clover or white clover silage compared to ryegrass silage. Higher voluntary intake of legumes is attributed to both a lower resistance of legumes to chewing and a higher rate of particle breakdown, digestion and clearance from the rumen (Waghorn *et al*., 1989; Steg *et al*. 1994).

Under grazing, the differences observed in forage yield and structure and management would affect the conclusion drawn from *ad libitum* fed animals. An experiment carried out over two years (13 rotations) managed at similar pasture allowance (22 kg DM per cow per day) clearly demonstrates advantage of MSS on a per cow basis for pasture DM intake and milk yield (Roca-Fernandez *et al*., 2014). The treatments were pure ryegrass, mixture of ryegrass and white and red clover, same mixture plus chicory and same mixture plus chicory and fescue. Pasture intake and milk yield were greater on the clover mixture than on pure grass (+1 kg per cow per day) and further increased by 0.5 kg on the more complex mixtures including chicory and fescue. In this experiment the total number of grazing days was hardly affected by the treatment, so that milk output per hectare was higher for MSS than for pure ryegrass swards (+ 1700 kg ha^{-1}). Similar results were obtained for grazing cattle (Morel *et al*., 2014) when comparing ryegrass and the mixture of four species. Several short term trials have previously shown that increasing the content of white clover in pasture increases milk yield by 1 to 3 kg per cow per day when pastures were compared at the same herbage allowance (Philips and James, 1998; Ribeiro Filho *et al*., 2003).

On low productive permanent grassland, a rotational grazing experiment was conducted in Germany over five years (2007-2011) using sheep and cattle in mono or mixed grazing of swards differing in diversity. Herbicides against dicotyledonous plants were used on plots in permanent grassland, resulting in a grass-dominated (gd) sward (6.9 ± 1.5 species m^{-2}) compared to the untreated diverse (div) sward (10.3 ± 2.9 species m^{-2}). Both diversity treatments were either grazed by sheep (S), or cattle (C). Grazing cattle were suckler cows and calves of the breed German Simmental. Ewes with lambs were Blackheaded and Leine sheep in comparable proportions of (C) or both (CS). Lamb production was slightly enhanced on the diverse swards and this effect was consistent over time, whereas calf performance was unaffected by sward type. Lamb growth was further improved by mixed grazing. These results

suggest that mixed, rotational grazing of cattle and sheep on phytodiverse agricultural swards is particularly appropriate to enhance lamb production (mixed grazing: + 17%; diverse swards: + 12%) without having any disadvantages for calf performance.

Potential for optimising the environmental roles of grassland through the concerted use of plant species diversity at the field level

Nutrient management is a main lever to improve the environmental performances of grassland-based production (Huguenin-Elie *et al.*, 2012). Notably, it is expected that reducing N fertilization while maintaining productivity thanks to the use of efficient, legume containing MSS could significantly modify the provision of regulating and supporting services from grassland-based ruminant production systems (Tilman *et al.*, 2002; Finn *et al.*, 2013). Different field experiments, including the CE, were performed to assess options for improving the environmental roles of grassland at the field level and to show that increased biomass productivity of grassland through the concerted use of legume-based MSS does not conflict with the delivery of a broad range of services.

Considering N use efficiency and losses affecting water quality, the use of legume-based MSS showed clear advantages compared to fertilizer-based grass monocultures. Mineral N (N_{min} including nitrate and ammonium N) was studied as large amounts poses a risk of pollution of ground water. Measurements of N_{min} indicate that MSS combining both N-fixing and non-fixing species and including a deep-rooting species performed as well in term of residual mineral N in the soil (N_{min}) as pure perennial ryegrass swards, and that they perform better in term of N_{min} per unit of DM yield, showing a lower risk of N losses for a similar yield. The results further show that moderately fertilized monocultures of the shallow rooting perennial ryegrass swards do not guarantee a low N_{min} content in the soil during winter, and that monocultures and mixtures with the deep-rooting red clover as N-fixing component perform better that those with the shallow rooting white clover (Figure 3). Three experiments (Wales, Ireland, Switzerland) considered the risk of nitrate leaching and showed that high-yielding forage production systems using symbiotic N_2 fixation and moderate levels of N fertilization do not increase the risk of nitrate leaching compared to grass monoculture systems relying on heavy N fertilization. Under cutting, the potential of nitrate leaching always remains very low (less than 1.5 mg nitrate l^{-1} water in ceramic cups) and the grass-legume mixtures did not show any elevated risk as compared to pure grass swards. Under grazing, only white clover was used as an N fixing legume. Nitrate losses from the grass-legume grassland were similar to losses from the grassland receiving higher inputs of fertilizer N and were directly proportional to the amount of N cycling within the systems regardless of the original source of N. A slightly higher risk of nitrate leaching was observed at two of the four studied points in time in the Teagasc site. These results fit well with previous reported data (Loiseau *et al* 2001).
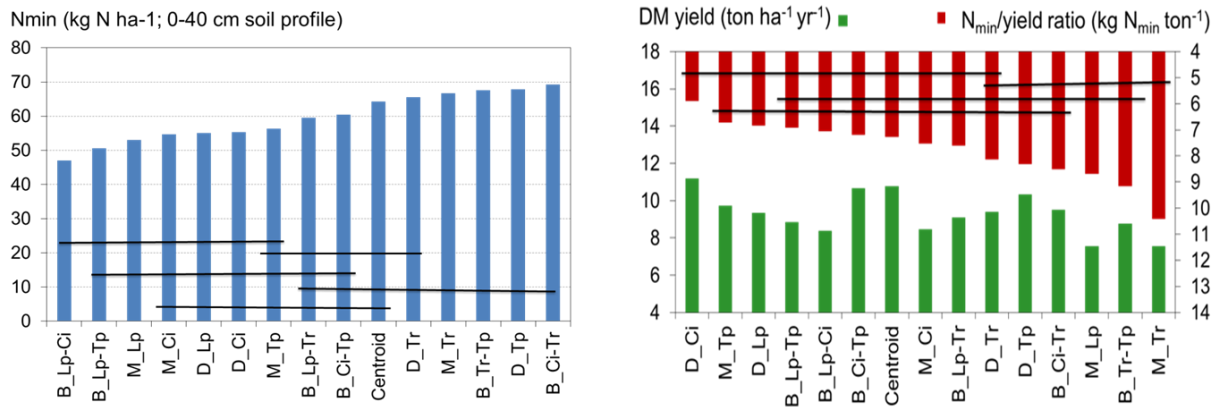
Figure 3: Effect of sward species composition on N_{min} in the 0-40 cm profile. Average of 1 site in Switzerland, 1 site in Wales and 2 sites in Poland from the common experiments sampled in the early winter and in the early spring. Horizontal lines join treatments that are not significantly different. D: 4-species mixtures with the indicated species being dominant, B: binary mixtures, M: Monoculture, Centroid: equal stand mixture combining the 4 species in equal amounts on a seed weight basis, Lp: *Lolium perenne*, Ci: either *Cichorium intybus* (Poland and Switzerland) or *Festuca arundinacea* (Wales), Tp: *Trifolium pratense*, Tr: *Trifolium repens*.

Considering the greenhouse gas (GHG) emissions impacting air quality, two experiments of MultiSward provided new information on N_2O and CH_4. Direct N_2O emissions from symbiotic fixation are considered negligible (IPCC, 2006) and, taking into account the fertilization-linked N_2O emissions (Nemecek *et al.*, 2001), it seems reasonable to consider that the partial replacement of N fertilization with symbiotic N_2 fixation from legumes is an effective way of lowering N_2O emissions from productive grasslands. Direct N_2O emission from symbiotic N_2 fixation was found to be negligible, and considering the quantity of N_2O emitted per unit of forage produced, grass-legume mixtures performed as well as or better than grass monocultures in the two experiments. For example in the Irish study, annual N_2O emissions from unfertilized mown perennial ryegrass-white clover plots and unfertilized mown perennial ryegrass were similar (2.38 vs 2.45 kg ha^{-1} year^{-1}). Under grazing there was an obvious trend of lower N_2O emissions from ryegrass-white clover plots compared to highly fertilized ryegrass plots, where annual N_2O emission were 15% higher (7.8 vs 6.4 kg N ha^{-1} year^{-1}) thus confirming the compilation of Jensen *et al* (2012). Legumes can contribute to reducing ruminal methane production (Waghorn *et al.*, 2006). Enteric methane emission per unit of feed intake was reduced in some MultiSward experiments, but not in all cases, depending on the forage and mixtures tested, and the animals. Enteric methane emission tended to be lower when pure legumes were fed indoors to sheep compared to pure grasses but the differences remained small (10%, Niderkorn *et al.*, 2014) and were negligible when considering grass/white clover with 10 to 50% clover in the mixture. It is noteworthy that methane production linearly decreases when increasing the proportion of chicory in a mixture of ryegrass and chicory (-2% per 10% increase of chicory in the mixture). In grazing dairy cows, methane emission per unit of feed intake was lower for grass/white clover cows compared to grass-only cows (21.5 vs 24.5 g kg^{-1} DM intake respectively). These results can be attributed to the differences between grass and clover or chicory during the digestion process, due to the high digestibility of white clover and the low fibre content of chicory, and thus to a modified ruminal fermentation pattern toward propionate (which is a hydrogen carrier) combined with an increased passage rate of legume particles.
Permanent grassland displays a strong potential for C sequestration (Soussana *et al.*, 2010). However, uncertainties surrounding estimates are often larger than the sink itself. Long term data on C flux and soil organic carbon in permanent grasslands for two grazed long term observation sites located in central France were analysed comparing a control (1.1 LU ha^{-1} with 213 kg N ha-1 year^{-1} and an extensive system (0.6 LU ha^{-1}, no fertilisation). The data confirm

the importance of multi species permanent sward for carbon storage (order of magnitude of 2 t ha^{-1} year^{-1}) but the sink activity is very variable. The most intensive system led to higher annual sink activity in years of dry and warm growing seasons (i.e. 2003, 2005, 2008 and 2011), whereas in years with more ample seasonal rain events (i.e. 2004, 2006, 2007 and 2010) the extensively grazed paddock held a higher sink activity (Figure 4). When taking g CH_4 and N_2O emissions in the net GHG balance, the sink activity of these ecosystems remained but was lower (order of magnitude of 2 t ha^{-1} year^{-1})
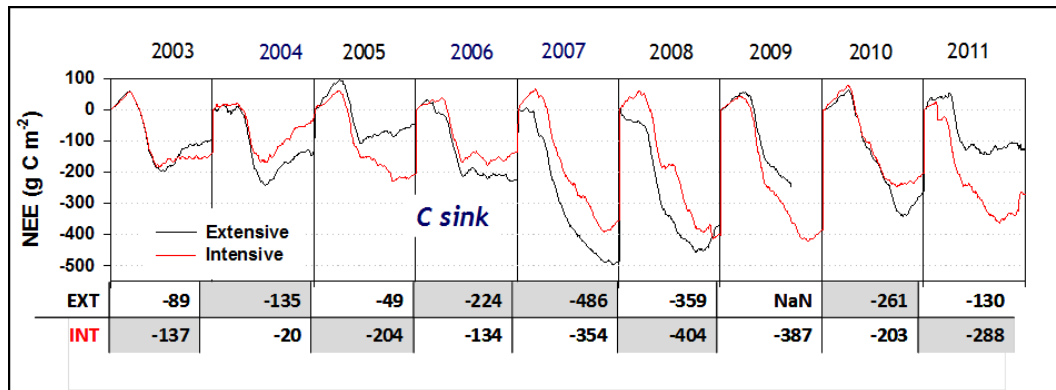
Figure 4. Cumulated net ecosystem exchange (NEE) measured from 2003 to 2011 for the extensively and intensively grazed paddock, with annual sums below.

Concerning aspects of soil functioning, one commonly proposed mechanism to reach functional complementarity in MSS is belowground vertical niche differentiation between shallow-rooting and deep-rooting species (Berendse, 1981; von Felten and Schmid, 2008). Beside this potential vertical niche differentiation, the difference in phenology between species may lead to different nutrient demand in time and therefore to a reduced completion for nutrient in multi-species communities. Both would result in a more complete exploitation of the soil and its available resources leading to higher above-ground biomass production. The utilisation of the soil profile was assessed using tracers (Hoekstra *et al*., 2013) on temporary grasslands sown with a range of botanical compositions. The studies showed that the proportional nutrient uptake from the shallow soil layer was significantly higher for the two shallow-rooting species (perennial ryegrass and white clover) compared to the two deep-rooting species (chicory and red clover), resulting in niche complementarity between shallow and deep rooting species. This is consistent with the study on residual N_{min} in the soil. Multi-species swards combining shallow- and deep-rooting species therefore efficiently use the soil profile. On the other hand, some evidence for temporal niche differentiation in N uptake from fertilizer (using ^{15}N) was found between perennial ryegrass (spring species) and red clover and chicory (summer species). Mixing species with different temporal patterns of nutrient uptake might therefore contribute to a high nutrient capture and consequent high biomass production in MSS.

Concerning non-renewable energy consumption to produce forage, legume-based MSS allowing high productivity with little fertilizer could greatly improve the consumed/produced energy ratio of forage production. Using the LCA methodology to asses this question from the yield data of the CE, it was shown that the 4-species grass-legume mixtures performed better than the grass monoculture at all five sites, (2.87 and 2.10 MJ-eq kg^{-1} DM respectively for the mixtures and the grass monoculture). Per kg of produced forage, production with the MSS required between 67 to 84% of the energy required with the grass monoculture at the same level of fertilization.

Grassland management as a lever to manage grassland biodiversity from the field to the landscape level

The beneficial ecosystem services provided by grassland partly depend on biodiversity, and the choice of plant and animal taxa that were considered accounted for this aspect. Because recent advances in ecology have stressed the benefits of not only considering plant taxonomic classification but also their functional classification for the study of ecosystem functioning, MultiSward tested the relevance of the functional approach for understanding the effects of management and climate on grassland diversity.

At the plot scale, the dependence of plant functional diversity criteria in permanent grasslands to management and climate was analysed using a large dataset from 439 permanent grasslands covering a large range of soil and climatic conditions and management gradient. A first data set contains the surveys of 140 permanent grasslands from all French regions apart from the Alps and Mediterranean area, a second one contains a survey of 70 permanent grasslands from the Vosges region and the third one consist in the survey of 229 permanent grasslands in the Swiss Alps. Plant species richness and the community-weighted value of Specific Leaf Area (SLA), Leaf Nitrogen Content (LNC) and of the onset of flowering (OFL) are the considered variables because they are involved in the delivery of many ecosystem functions and services such as forage quantity and quality, or pollination (Lavorel *et al.*, 2011). Links between climatic and management variables on species richness were analysed using a regression tree approach to select the most important variables. More than 60% of the variance in species richness could be explained by the surveyed climatic and management variables. Plant species richness and the onset of flowering increased with altitude, while the community-weighted mean of SLA and LNC decreased with altitude. Both defoliation intensity and N inputs had positive effects on SLA and LNC which confirms the results of previous surveys (Lavorel *et al.*, 2011). Defoliation intensity had a negative effect on the onset of flowering, which could be related to plant reproductive strategy, since an early onset of flowering would allow species to complete their reproductive cycle before being defoliated. The climatic variables generally influenced species richness more than management variables, from which total N input and intensity of defoliation were the most important variables and the type of utilisation (grazing vs mowing) had a weaker effect. The management variables that appeared to exert the strongest effect on plant species richness differed along the climatic gradient. The response of the number of species to management intensity was different between the grasslands from regions with a colder climate and the ones from regions with a warmer climate. Conditional effects of management on species richness were also observed by de Bello *et al.*, (2006). The intensity of defoliation, which generally produces a negative effect of the number of species (Gaujour *et al.*, 2012), appeared as a main lever of the within-plot species richness (alpha diversity) within the regions with a rather cold climate, but not within the regions with a warmer one. Increasing applications of mineral or organic fertilizer generally result in a drop in plant species richness (Schellberg *et al.*, 1999). In our dataset fertilization appeared to be a factor strongly influencing species richness, but especially for the warmer grasslands with summer rainfalls of more than 200 mm. Grazing was found to be positive for the number of plant species per plots only in two regions. These results show that strategies targeting an increase in within-plot species richness by modifying management practices need to be developed at the scale of small regions.

At the farm scale, the effect of heterogeneity among grasslands has nevertheless seldom been quantified, although it is widely recognized that habitat heterogeneity has a positive effect on biodiversity in agricultural landscapes (Benton *et al.*, 2003). Grasslands managed differently within a farm can shelter different plant communities with to some extent different species, which could have a positive effect on the overall species richness. An analysis was based on a dataset consisting in the survey of permanent grasslands in the Swiss Alps (235 grasslands-Grindelwald region) with model farms having 20 different grassland plots and different

management strategies based on four management classes (intensive vs. extensive and low vs. high altitude) and additional dataset consisting in the survey of 69 grassland plots from 9 farms from Norway and 31 farms from French Jura. The results consistently show that most of the richness in plant species richness is due to the between-plot diversity (β-diversity; Figure 5).
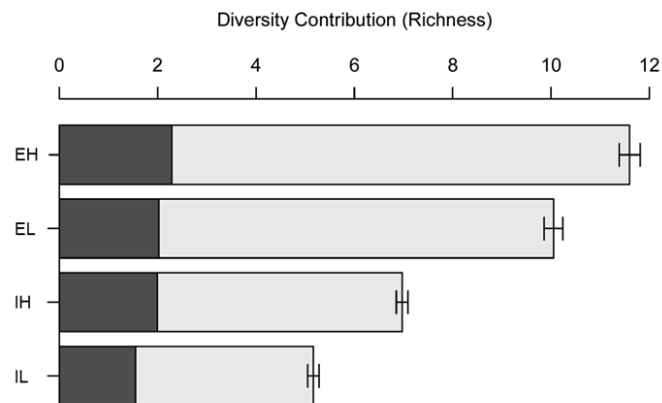

Figure 5: Average diversity contribution per plot of different management classes for the alpha, beta and gamma diversity (richness) at the farm level for the Grindelwald region and for a farm with 5 plots of each management classes. Dark grey: alpha contribution; light grey: beta contribution; total: gamma contribution; standard error shown for gamma contributions only. IH: intensive management, high altitude, EH: extensive high, IL: intensive low altitude: EL: extensive low

As this β-diversity is large within all types of management of permanent grasslands, farms with many plots always have much larger species richness at the farm scale than the average α-diversity of their plots. Both a larger α- and a larger β-diversity was found within the sets of extensively managed plots than within the sets of more intensively managed ones. Heterogeneity between grasslands therefore proved very important for plant species richness at the farm level. Promoting heterogeneity thanks to a differentiated grassland management at the farm scale thus appears to be an important component of diversity conservation in productive ruminant production systems located in grassland dominated landscapes. Such a strategy should mainly consider supporting infrequently mown grasslands receiving no or little nutrients and situated in less favourable locations, as well as extensively grazed grassland.

Considering fauna diversity at plot level, it still remains difficult to assess the effect of grassland management and plant diversity on the abundance and diversity of various taxa. MultiSward developed a set of indicators to evaluate the impacts of grassland plant diversity and management on the abundance and diversity of six animal taxa selected due to their contrasting biological requirements and key biological functions (e.g., pollination, pest control, soil fertility). The methodology combines multi-criteria decision trees to predict taxa diversity according to management practices, sward composition and plant functional traits with fuzzy partitioning, allowing assessment of different types of information (qualitative or quantitative, more or less accurate knowledge) and which makes possible a more precise assessment than the DEXI one, which only permits propositions having a value of truth or falsity. Decision trees aimed to predict taxa diversity (Plantureux *et al.*, 2014). First results obtained on fauna biodiversity at plot level reveal the usefulness of including plant diversity and simple management inputs to improve the environmental evaluation of grassland-based systems. Plant species richness appeared to have a direct and positive effect on butterfly and moth diversity, and on grasshopper species richness. Bumblebee abundance was also positively related to legume abundance, and the abundance of erect growth-form plants was assumed to have a major effect on the diversity of web-building spiders. Finally, it is noteworthy that the abundance of grasses strongly influenced earthworm abundance, which suggests that earthworms might be the only group that would not take advantage of an increase in sward diversity. For all these

taxa grassland plant diversity and an adequate management can preserve a high level of biodiversity.

Innovations in grazing management to increase competitiveness and environmental benefits of grassland based systems

The challenge for farmers in the years ahead is to increase the competitiveness of their business through innovation, productivity gain and increased operational scale with revenue (at least for milk production) projected to fluctuate/fall and the cost of production increasing. Achieving an efficient use of grassland is a key issue in this context.

Extending the grazing season to increase pasture utilisation

Several experiments have shown there is considerable opportunity to extend the grazing season in early spring and late autumn in intensive dairy systems in the west part of Europe (Dillon *et al.*, 1998; Peyraud *et al.*, 2010). Extending the grazing season reduces the requirement for silage, purchased feedstuffs, housing, and slurry storage and spreading, thereby improving the economic returns to the producer from their ruminant production system. In continuation of this work, MultiSward tested this practice in the case of more extensive systems in central Europe, examining strategies that can be incorporated into grazing systems in autumn and spring with respect to concerns about nitrate leaching.

As previously demonstrated in Western Europe, extending the length of the grazing season is also feasible in nutrient poor grassland in central Europe. In the western part of Poland, the extension of the grazing season for suckler cows until the end of the year is possible without adverse effects on animal welfare (Piatkowski *et al*, not published). Paddocks were grazed in August and then closed until grazing in late October, November or December by 5 cows (Angus and Angus×Limousin; BW 460 to 520 kg). Herbage intake decreased during the season. Climatic conditions in Central Europe fluctuate much more than in the Atlantic climatic zone. This is reflected in fluctuations in the yield of the pasture sward between years and months (from 1.7 to 3.5 t ha^{-1}), its utilization rate (from 65 to 81%) and animal intake (from 5.6 to 11 kg DM cow^{-1} per day). Good availability of herbage and favourable grazing conditions occurred in November 2011 and the poorest conditions in November 2012. The sod damage in each paddock, as a consequence of suckler cows grazing was low with the exception of November 2010, when heavy rainfall occurred (133.8 mm).

Restricted access time to pasture can be used as a strategy to increase the length of the grazing season while minimizing damage to pasture by poaching during periods of inclement weather. In spring, restricted access time to pasture has been shown to have no (Kennedy *et al.*, 2009) or a slightly negative (Perez-Ramirez *et al.*, 2008) effect on milk yield. An experiment conducted in Ireland has examined the effect of restricted access to pasture in the autumn of late-lactation spring-calving dairy cows and showed that that restricted access time to pasture can be implemented on dairy farms in autumn with no reduction in dairy cow production. Forty-eight cows were assigned to one of four treatments: full-time access to pasture, two 5-hour periods of access to pasture after a.m. and p.m. milking, two 3-hour periods of access to pasture after a.m. and p.m. milking, and alternating between full time and 2x3H access to pasture with no more than three continuous days on any one regime. Treatment had no effect on animal performances (milk solid yield (1.15 kg day^{-1}) or herbage intake (15 kg day^{-1})). This was due to changes in the cows' grazing behaviour as, when access time to pasture was restricted, the grazing intensity increased leading to higher intake per minute and per bite.

Removal of herbage and poaching as a result of grazing in autumn and the low utilisation of urine and faeces during the late autumn due to low grass growth rates increases the possibility of leaching and runoff. This was addressed by two experiments showing that the risk of nitrate leaching is not severely increased by extending the grazing season length and can be brought under control. In Ireland, the effect of nine grazing season lengths on nitrate leaching to 1 m in

the soil was tested using ceramic cups. Ceramic cups were sampled on 56 occasions between 25 January 2007 and 15 June 2010. Cows were turned out to grass post calving on 1 February, 21 February or 15 March, and remained at grass fulltime until 10 October, 25 October or 10 November. The same stocking rate and fertilisation levels were applied (2.47 LU ha^{-1} and 250 kg N ha^{-1} year^{-1} respectively). Nitrate leached ha^{-1} was similar for the three autumn housing dates (152 kg N-NO$_3$ ha^{-1}). However, management of paddocks affected nitrate leached: the control treatment (no grazing or fertiliser N) had the lowest level (36 kg N-NO$_3$ ha^{-1}), the grazing-only management had the highest (181 kg N-NO$_3$ ha^{-1}), and paddocks that were grazed and had silage harvested from them had intermediate values (109 kg N-NO$_3$ ha^{-1}). In Belgium the effect of cutting in autumn was examined in more detail. The nitrate content in the 0-90 cm soil horizon was compared between plots either cut (one or two) or grazed from 1 September until the end of the growing season in nine pastures on three soil types (sand, sandy loam and clay) over three years. The evolution of soil nitrate content in the period 1 September – 15 November was not dependent on the grassland management, cutting instead of grazing having the same effect on the nitrate content in the soil profile. However there was high variability in soil nitrate content on 1 September within pastures intensively grazed during the growing season. Taking 2 cuts – end of August and middle of October - can decrease the nitrate N-content in the soil at the end of the growing season in comparison with grazing, but taking only one cut (in the middle of October) has less potential to do so (respectively 88, 71 and 50 kg N-NO$_3$ ha^{-1}). The N-uptake by the grass in autumn, under a 1- and 2- cut regime was considerably different (144 vs 98 kg N ha^{-1} for 2 cuts and 1 cut respectively).

Grazing management in uplands pastures designed to increase biodiversity

For upland pastures one of the challenges is to manage a high level of biodiversity, while developing appropriate grazing management strategies can contribute to increased grassland biodiversity. An experiment evaluated the consequence of withdrawing animals during the main flowering period on the biodiversity. An 'ecological rotation' strategy, taking the animals away from one rotational subplot during the main flowering period to decrease the stocking density locally, thereby favouring flowering intensity, was compared with continuous grazing under cattle and sheep, and the effects on the abundance of bumblebees, butterflies and ground beetles in both grazing management systems was examined. Cattle grazed plots had a larger flowering cover than sheep grazed plots and the ecological rotation with sheep allowed better flowering cover than continuous grazing management; no difference was found in cattle grazed plots. Flowering, visiting insect density and species richness correlated positively with the flower cover of the plot. Butterflies and bumblebees benefitted from the ecological rotation management both in cattle and in sheep grazing but the benefit seemed weaker with sheep than with cattle grazing*,* sheep grazing, leading to lower butterfly and bumblebee abundance and lower species richness than cattle grazing. Managing the ecological rotation with cattle instead of sheep allowed an increase of 32% in the butterflies per transect, 28% in the butterfly species per transect, 61% in bumblebee abundance and 53% in bumblebee species richness.

Appropriate animal for successful temperate grassland-based systems

Apart from grassland management, the most profitable genotype or breed is a key factor to return the highest profit per unit of the most limiting input. For example it is now established that that cows selected solely on the basis of milk production have poorer fertility performances and are not well suited for grassland based systems (Dillon *et al*., 2003; Horan *et al*., 2005). The development of sustainable grassland-based ruminant systems clearly requires the most adapted animal genetics. In MultiSward, long term experiments were conducted to evaluate genotype x management interaction. Modelling data using the Moorepark Dairy Systems Model (MDSM) (Shalloo *et al*., 2004) from these long term grassland based dairy cow production systems allowed a more holistic evaluation of the systems, including production, some

economic and some environmental evaluation. On- and off-farm GHG emissions from dairy production were assessed using a cradle to farm-gate attributional LCA sub-model (O'Brien *et al.*, 2012).

A French experiment compared Normande (No), which is a dual purpose breed, with Holstein-Friesian (Ho) on High and Low input feed systems. Cows grazed from April to October. During lactation the High Input dairy cows consumed 1700, 2900 and 1450 kg DM cow[-1] of conserved forage, grazing forage and concentrate, while the Low Input cow consumed 1600, 3600 and 90 kg DM cow[-1] respectively. The response to feeding strategy was greater for Ho cows (-1325 kg milk, -168 kg milk solids) than for No cows (-117 kg milk solids) and the reproductive performance was highly altered for the Ho cows, with low gestation rate especially in the Low feeding group. Regardless of feeding strategy, no cows had lower BCS loss in early lactation and higher BCS at dry off than Ho cows. These results clearly showed the high reactivity of the milk production in Holstein cows to feeding level, which does not limit the body condition loss as well as the degradation of the reproduction performance, making the Holstein cow incompatible with low inputs systems especially when compact spring calving is required. On the contrary, the dual purpose breed appears more flexible and better adapted to low input systems based on the maximisation of grassland use for milk production. The Ho breed had the lowest carbon footprint of milk. However, the relative difference between Ho and No breeds was low (1.25 vs 1.32 kg eq CO_2 t[-1] milk respectively for Ho and No) and varied according to the allocation methods between milk and meat due to the value for surplus calves and culled cows. Briefly, regardless of feeding system, the Ho and No breeds are similarly profitable in a 2005-06 prices scenario but with the 2007-08 scenario (high cereal prices) the Ho breed was more profitable than No. This was largely due largely due to the fact that fewer cows and heifers are required for the same quota due to the higher milk yield of the breed and so less land is required for grass. The additional land for the Holstein breed is then converted to cereal crops and the higher the cereal price, the greater the benefit associated with intensification of the milk production system

In Ireland, the biological efficiency of three genotypes (Jersey, Holstein-Friesian and Jersey × Holstein-Friesian) was compared across three grassland-based systems. The Holstein and JexHo animals were stocked at 2.5, 2.75 and 3.0 cows ha^{-1} while the Jersey animals were stocked 0.25 cows ha^{-1} higher for all treatments. Crossbred dairy cows are capable of production levels per cow at least similar to their Holstein-Friesian contemporaries on low cost systems (453 kg milk solids cow[-1]) but fertility and survival levels are markedly improved (e.g. six week in-calf rates were increased by over 10 percentage units with crossbreds). Jersey cow a lower yield (424 kg cow[-1]) but yield per hectare was not affected by the type of cows (1166 kg ha^{-1}). In Ireland, the impending removal of EU milk quotas will result in land becoming the most limiting resource. Economic analysis demonstrates a substantial profit benefit per lactation with the F1 cows. The difference in performance equates to over €12,000 annually on a 40 ha farm. This result is primarily attributable to improvements in milk revenue and the large differences in reproductive efficiency/longevity observed with the crossbred herds although lower cull cow and male calf values, especially during periods of high beef value, negate the potentially larger benefits from this cross. The Je×Ho cross also had a lower carbon footprint of milk than the Ho breed (4-10%) The range of the difference varied according to the allocation methods between milk and meat due to the value for surplus calves and culled cows.

Concerning meat production, a Polish experiment examined the efficiency of lamb production of four sheep breeds (White-headed meat sheep, Wielkopolska sheep, Romanov sheep, Blanc du Massif Central sheep) in continuous grazing conditions on lowland pasture. The results show that Romanov Wielkopolska sheep had similar live weight gain for the grazing season. It was mainly influenced by high live weight gain in May for Romanov whereas Wielkopolska sheep

had the highest live weight gain in summer when the pasture yield was greatest. Lamb live weight gain of white-headed meat sheep and Blanc du Massif Central sheep was lower.

Conclusion

MultiSward has provided science-based information and expertise that are of the utmost practical relevance for farmers and for EU agricultural policy for maintaining grassland acreage and developing competitive, productive and environmentally friendly livestock systems. Among the most promising outputs of the project, it is noteworthy that MultiSward has (i) provided a state-of-the-art review about the roles and utility of grassland and stakeholders expectations; (ii) provided useful information and pointed out the need for improving European statistics on grassland acreage and grassland term definition to design more efficient public policies for maintaining grassland acreage; (iii) demonstrated that margins of progression exist: clearly multispecies swards can contribute to more sustainable ruminant production systems; performance of grassland based system requires well suited breeds and appropriate grazing management strategies and (iv) provided adequate tools to assess the performances of production systems at different scales and for various territories.

Future programmes developing integrated approaches with multi-scale and multicriteria approaches are still necessary to improve our knowledge and to propose innovations for more competitive and sustainable grassland based systems. Some key research questions remain to be solved: (i) strategies for maintaining a functional legume presence (in terms of biomass) over time under both cutting and grazing managements. The issue of decreasing sward legume content was particularly evident in the case of red clover, but it was also apparent in white clover; (ii) in the context of climate change, the development and testing of new plant production systems and new multispecies grasslands having fewer requirements for water and higher resilience to dryness is required; (iii) progress is still required to determine the most appropriate ruminant phenotype and appropriate indicator traits that reflect improved forage use efficiency and reduced ecological footprint, and to fully exploit the adaptive capacity of herbivores to make better use of grassland in marginal land (land on which the only thing that will grow is grassland). Beyond research, a key issue for the future of grassland is to convince farmers to continue to use grassland and to help them to progress technically and economically. It would be particularly interesting to build a European grassland network (based on a so-called multi-stakeholder approach) aimed at: informing farmers and all relevant stakeholders; identifying, sharing and adopting innovations; and proposing references and demonstrations to improve the performance of grassland based systems and increase farmer confidence in these systems.

Acknowledgement

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Theme 5 submitted papers

Biomass production in multispecies and grass monoculture swards under cutting and rotational grazing

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Abstract

A Common Experiment (CE) was set up within the EU-FP7 project 'Multisward' across a subset of partner sites to analyse responses of multispecies swards (MSS) to grazing and cutting managements. Across sites and managements there was no detriment to yield in moderately fertilized legume-based MSS compared with perennial ryegrass monocultures receiving high inputs of external nitrogen fertilizer. The response of sward types to grazing depended on sward composition and the identity of the grazing animal species.

Keywords: grazing, legumes, multispecies mixtures, perennial ryegrass, yield

Introduction

Strategically designed multispecies swards (MSS) could be a key element in improving the delivery of provisioning services from grassland-based production systems (Finn *et al.*, 2013). A Common Experiment (CE) was set up within the EU-FP7 project 'Multisward' across a subset of three partner sites to analyse responses of MSS compared with highly fertilized perennial ryegrass (PRG) monocultures to grazing and cutting managements under temperate maritime (Aberystwyth (UK) and Rennes (FR)) and continental (Tänikon (CH)) environmental conditions. The CE imposed contrasting defoliation managements (grazing and cutting) typical of intensive production systems for 2-3 years on sward types differing in species number and composition. Differences in biomass production between sward types within each management were analysed. Within each sward type, grazed and cut plots received the same external applications of nitrogen fertilizer (N) and were defoliated at the same frequency to the same residual height, so that differences in sward responses to cutting and grazing managements could be attributed directly to the influence of the grazing animal. The effect of defoliation management on sward type was also analysed.

Materials and methods

Four forage species in common agronomic use in Europe were included in the CE: two grasses (perennial ryegrass - PRG; tall fescue – FA) and two legumes (white clover –WC; red clover – RC). The second grass was replaced with chicory (Ci) in Rennes and Tänikon. Over all sites the CE included MSS treatments (two legumes + PRG; two legumes + two grasses; two grasses; two legumes + two grasses + Ci) and monoculture swards of PRG, a subset of which received a 'high' application of N. All MSS received a 'moderate' application of N (150 kg N ha^{-1} yr^{-1} in Aberystwyth and Tänikon, and 70 kg N ha^{-1} yr^{-1} in Rennes). High-N PRG monocultures received 300 kg N ha^{-1} yr^{-1} in Aberystwyth, 350 kg N ha^{-1} yr^{-1} in Tänikon and 165 kg N ha^{-1} yr^{-1} in Rennes. Biomass production was measured during 2011-2013 in Tänikon (18 harvests), and during 2012-2013 in Aberystwyth (12 harvests) and Rennes (13 grazing and 10 cutting harvests).

Grazing management: Management was based on the concept of biomass accumulation and removal, as in rotational grazing systems. Different animals were used in the three sites: non-lactating ewes in Aberystwyth; dairy cows in Rennes; beef heifers in Tänikon. In each grazing interval, plots were grazed to a target sward height appropriate for the animal (5 cm in

Aberystwyth; 4 cm in Rennes; 6 cm in Tänikon), and the animals were then removed. The length of the regrowth period between grazing intervals was defined for each site and adjusted if necessary to take account of variation in forage production in response to climatic conditions. Biomass productivity in grazed plots was measured immediately prior to grazing by cutting the herbage in known areas within the plot to the target sward height. These samples were oven-dried and weighed.

Cutting management: Cut plots had the same species composition as grazed plots, with the same level of replication. In Tänikon these were fixed subplots within the grazed plots; in Aberystwyth they were randomized in a separate block beside the grazed plots; in Rennes cutting was only carried out on fixed subplots (high and moderate N) within PRG monocultures. Cut plots were mechanically defoliated, and biomass productivity was measured by subsampling the cut herbage, drying and weighing.

All sward type treatments were randomized and replicated three times, except for high-N PRG-monoculture plots, which were replicated four times. Results for biomass production were analysed by appropriate ANOVA structures (Rennes and Tänikon) and by REML (Aberystwyth). Preliminary results from these analyses are presented here.

Results and discussion

To integrate the effects of time, biomass productivity was expressed as cumulative dry matter (DM) yield. Results for total yield (sown + unsown species) are shown in Table 1.

Table 1. Cumulative total DM yields (sown + unsown species; kg ha^{-1}) over 2/3 years in three sites of the Multisward CE. Sward types: 1_M = PRG mono; 2 = 2 non-legumes; 3 = 2 legumes + PRG; 4 = 2 legumes + 2 non-legumes; 5 = 2 legumes + 3 non-legumes; 1_H = PRG mono receiving high N.

(a) Aberystwyth (12 harvests)

Sward type	1_M	2	3	4	5	1_H	*Mean*
Management							
CUT	15510	15683	27089	25073	-	17459	*20163*
GRAZED	12852	11944	19229	20865	-	17531	*16484*
Mean	*14181*	*13814*	*23159*	*22969*		*17495*	

(b) Tänikon (18 harvests)

Sward type	1_M	2	3	4	5	1_H	*Mean*
Management							
CUT	17382	21769	24491	26678	-	22905	*22645*
GRAZED	20423	24363	28989	31651	-	29718	*27029*
Mean	*18903*	*23066*	*26740*	*29164*		*26312*	

(c) Rennes (13 grazing harvests; 10 cutting harvests)

Sward type	1_M	1_M	2	3	4	5	1_H	*Mean*
Management								
CUT	23865		-	-	-	-	26352	*25109*
GRAZED	22901 *	26520	-	28590	29481	29011	-	*28401*
Mean	*23393*							

Note: Two non-legume species in Aberystwyth = PRG and FA; in Zürich and Rennes = PRG and Ci.
Sward type 5 = 2 legumes + PRG + Ci + FA. * Yield calculated from 10 harvests to allow direct comparison with cut treatment

Aberystwyth: There were significant effects of management and sward type ($P<0.001$), together with an interaction between them ($P<0.047$). Overall, cumulative yield was higher under cutting than grazing (20163 vs. 16484 kg ha^{-1}). Sward types 3 and 4 were the highest-yielding swards, overall. The management × sward type interaction resulted from a large difference in response between legume-based and non-legume-based MSS to defoliation. Yields of the legume-based sward types 3 and 4 were significantly higher under cutting than under grazing, whereas yields of the grass-based treatments 1_M, 1_H and 2 were not affected by management.

Tänikon: There was a significant effect of management averaged over sward type ($P<0.008$), and cumulative yield under grazing was higher than under cutting (27029 vs. 22645 kg ha^{-1}). Averaged over managements, the effect of sward type was significant ($P<0.006$). Sward types 3, 4 and 1_H were the highest yielding, and 1_M was the lowest. There was no management × sward type interaction, so all sward types responded to defoliation in a similar way.

Rennes: The experimental design at this site reduced the number of sward types under cutting. Within this management there was a significant difference between high and moderate N PRG monocultures ($P<0.007$), the former being more productive. There was no effect of defoliation management on cumulative yields of the 1_M treatment. Under grazing there was no difference in the yields of the various sward types.

The agronomic utility of MSS was tested here by comparing the yields of sward types under realistic defoliation managements. We observed different sward responses to defoliation management in different sites. However, there was a confounding effect of the use of different grazing animals at each site (although this aspect of the CE also added to its agronomic relevance). In Aberystwyth, grazing either had no effect on sward yield (grass-based swards), or reduced it (legume-based swards) compared with the cutting management. Sheep grazing appeared to have a direct and detrimental effect on the legume component of MSS. In Tänikon, cattle grazing had a positive effect on yield in all sward types and there was no interaction of defoliation management with sward type. Thus, the most productive swards under grazing would be also the most productive under cutting in this site. Rennes carried out only one cut *vs.* grazed sward-type comparison, in which there was no effect of defoliation management on yield. Taken together, these results support the hypothesis that selective grazing can have a large effect on the yield of MSS, and that the identity of the grazing animal is the key determinant. It is well known that species of grazing animal differ in their ability to select sward components. Yarrow and Penning (1994) found that the proportion of white clover in mixtures was lower when they were grazed by sheep than by cattle, both of which were lower than if the swards were harvested by cutting. Our results show that there was no detriment to yield in legume-based MSS compared with high-N PRG monocultures, and in some instances MSS were more productive. Increased use of MSS therefore potentially represents a substantial economic and environmental saving when the various costs associated with the use of nitrogen fertilizer are considered.

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Yarrow N.H. and Penning P.D. (1994) Managing grass-clover swards to produce different clover proportions. *Grass and Forage Science* 49, 496–501.

Nitrogen capture in mixed swards benefits from temporal complementarity among species

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Abstract

Highly fertilized grass monocultures carry a risk of nitrogen (N) losses to the environment. Studies have shown that grass-legume swards help to reduce the need for fertilizer applications for similar levels of biomass production due to their access to nitrogen from the atmosphere. However, other functional traits, such as root architecture and differences of phenology in time, may influence N uptake. Our study aimed at assessing which combinations of functional traits allow large N acquisition and high biomass production. Four forage species with different belowground traits (N_2-fixing/non N_2-fixing; shallow/deep rooting) were sown as monocultures and as mixtures of two or four species. Recovery of fertilizer N in the harvested plant biomass was measured during one regrowth in spring and one in summer using ^{15}N labelled fertilizer. Productivity and ^{15}N recovery were correlated. The recovery of fertilizer N was not reduced by the presence of N_2-fixing species grown in productive mixtures. No clear effect of rooting depth on the acquisition of fertilizer N was observed, although monocultures of shallow-rooting species performed poorly both in terms of yield and of fertilizer N recovery. In spring, ^{15}N recovery was highest with mixtures containing *Lolium perenne,* whereas in summer it was highest with swards containing *Trifolium pratense* and/or *Cichorium intybus*. In an average of both seasons, combining *L. perenne* with one or both of these summer species allowed a large ^{15}N recovery and biomass production at each period of regrowth, which indicate benefits of temporal complementarity for N uptake.

Keywords: ^{15}N recovery, temporal complementarity, multi-species swards

Introduction

Intensive forage production based on grass monocultures and high nitrogen (N) fertilization carries a risk of N losses to the environment. Grass-legume mixtures have been suggested as an alternative to this system. Optimizing mixtures for high yields and complementarity in nutrient acquisition between species might increase efficiency of nutrient capture: Nyfeler *et al.* (2011) showed that the uptake of soil and fertilizer N can be higher in mixed swards combining 60% of grasses and 40% of legumes than in grass monocultures. Despite the high percentage of N derived from symbiotic N_2 fixation, these grass-legume mixtures produced such a high yield that their demand on non-symbiotic N was large. Most studies on nutrient uptake in intensively managed mixed swards have focused on grasses and legumes. In addition to grass and legume species, *Cichorium intybus* was added in our experiment. This species is a non-N_2 fixing species with a deep tap-root system, which differs from the grass root system. Such differences in root architecture might reduce competition for N within the soil profile, increasing the total N uptake of the community (Berendse, 1982). In addition, differences in phenology may induce asynchronous N demand between the species. This temporal complementarity between species may reduce competition for nutrients (Casper and Jackson, 1997). Our study aimed at determining which combination of plant traits (spatial and temporal complementarity) allows for high N acquisition from fertilizer and high biomass production in frequently defoliated multi-species swards.

Material and methods

Four replicates of eleven types of swards, based on four forage species differing in belowground traits, were sown in April 2011. These species were: *Lolium perenne* (non-N₂ fixing and shallow rooting species), *Cichorium intybus* (non-N₂ fixing and deep-rooting species), *Trifolium repens* (N₂ fixing and shallow-rooting species) and *Trifolium pratense* (N₂ fixing and deep-rooting species). The eleven types of swards included the monoculture of each forage species, all six combinations of two of the four species, and the four-species mixture with species sown in equal relative abundance. The swards were harvested six times per year and fertilized with 145 kg N ha^{-1} year^{-1}. Two weeks before the harvests in May 2012 and July 2012, 0.64 L of a solution of ^{15}N labelled fertilizer (ammonium nitrate; 0.03 g ^{15}N m^{-2}) was applied with a watering can on 0.64 m^2. At harvest, a plant sample was cut at 6 cm in this subplot to measure ^{15}N content in the plants. The ^{15}N recovery (%) in the harvested biomass was calculated using the following equation: $\frac{^{15}N_{plant} - {}^{15}N_{natural}}{^{15}N_{fertilizer}} x100$

where $^{15}N_{plant}$ is the amount of ^{15}N (g m^{-2}) contained in the biomass harvested from the labelled area, $^{15}N_{natural}$ is the amount of ^{15}N in plants harvested in an unlabelled subplot and $^{15}N_{fertilizer}$ is the amount of ^{15}N applied with the fertilizer. An ANOVA was performed to compare the differences in ^{15}N recovery and biomass production between the types of sward.

Results and discussion

Between 9 and 25% of the applied ^{15}N were recovered in the plant biomass above 6 cm, two weeks after the application of the labelled fertilizer (Table 1).

Table 1. ^{15}N recovery in the harvested biomass at the end of the period of regrowth in May and July 2012 and annual yield in 2012. Each value corresponds to the mean of four replicates. Within a column, a common letter indicates no significant difference between types of swards. Within a line, ^{15}N recovery did (*) or did not (ns) differ between May and July 2012.

Types of swards		^{15}N recovery (%)				Annual yield (g m^{-2})
Root. depth	N₂ fixation	May 2012	July 2012	Δ July-May	mean	
	non-N₂ fixing					
Shallow (s)	*Lp*	16.4 abc	11.2 ab	-4.8 ns	14.0 bc	709 d
Deep (d)	*Ci*	16.5 abc	25.0 a	8.5 *	20.8 a	976 bc
s and d	*Lp-Ci*	24.4 a	16.7 ab	-7.7 *	20.6 ab	929 bc
	N₂ fixing					
Shallow	*Tr*	9.8 c	10.4 b	0.7 ns	10.1 c	669 d
Deep	*Tp*	10.6 bc	21.7 ab	11.0 *	16.1 abc	1190 a
s and d	*Tr-Tp*	10.3 bc	19.1 ab	8.8 *	14.7 abc	1225 a
	Mix(fixation)					
Shallow	*Lp-Tr*	21.0 a	13.7 ab	-7.3 *	17.3 ab	820 cd
Deep	*Ci-Tp*	8.9 c	22.4 ab	13.6 *	15.7 abc	943 bc
s and d	*Ci-Tr*	14.9 abc	21.1 ab	6.1 *	18.0 ab	901 bc
s and d	*Lp-Tp*	20.7 ab	16.8 ab	-3.9 ns	18.7 ab	1077 ab
s and d	Equal stand	20.7 ab	19.4 ab	-1.3 ns	20.1 ab	1156 a

^{15}N recovery was not generally smaller with the swards containing only N₂ fixing species than with those containing only non-N₂ fixing species (lower ^{15}N concentration in the plants but larger yield; data not shown). In accordance, ^{15}N recovery was not reduced by the presence of N₂-fixing species in mixtures (*P*>0.05). No clear effect of rooting depth on the acquisition of fertilizer N (^{15}N recovery) was observed. On average over both seasons, ^{15}N recovery was generally lower with the monocultures of shallow-rooting species (*L. perenne* and *T. repens*)

than with the other swards. This could have been an effect of poorer growth, as biomass production was significantly lower in these two monocultures.

In May, a higher ^{15}N recovery was achieved in the *L. perenne* and *C. intybus* monocultures than in the *T. repens* monoculture. While ^{15}N recovery did not significantly differ between seasons with the *L. perenne* monoculture, it significantly increased between May and July with the *C. intybus* monoculture. There was also a difference in the dynamics of ^{15}N recovery between the two *Trifolium* species: in July, ^{15}N recovery remained low in the *T. repens* monoculture, whereas it significantly increased between May and July in that of *T. pratense* (Table 1). Thus, with respect to the capture of fertilizer N, *L. perenne* may be considered a spring species and *C. intybus* and *T. pratense* summer species. This different temporal pattern between species might explain why mixed swards containing *L. perenne* allowed a larger ^{15}N recovery than the others in May (swards with *vs*. without *L. perenne*: $P<0.05$) and that mixed swards containing *T. pratense* and/or *C. intybus* allowed a larger ^{15}N recovery than swards without these species in July (swards with *T. pratense*/*C. intybus vs*. without: $P<0.05$). The lowest ^{15}N recovery on average from both seasons was found in the *T. repens* monoculture, whereas the combination of spring and summer species led to a larger ^{15}N recovery than other swards (spring-summer swards *vs*. other swards: $P<0.05$). Mixing species with different temporal pattern of fertilizer N capture, such as *L. perenne* (spring species) in combination with *T. pratense* and/or *C. intybus* (summer species), might reduce the competition for N during a given period of regrowth (Casper and Jackson, 1997) and lead to high total N uptake within the whole growing season. Recovery of fertilizer N was, nevertheless, equal in the monoculture of *C. intybus* as in the spring-summer species mixtures. As fertilizer acquisition and biomass production are correlated (May: $P<0.05$, $R^2=0.60$; July: $P<0.05$, $R^2=0.51$), swards combining spring and summer species conserved high fertilizer uptake and biomass production at each period of regrowth. That may contribute to stabilization of the productivity of swards within a year (Wayne Polley *et al.*, 2007) and lead to a high annual biomass production in these swards (Table 1).

Conclusion

Recovery of fertilizer N was not reduced by the presence of N_2-fixing species in multi-species swards. Mixtures with *T. pratense* achieved high yields and therefore had a high demand for nitrogen. No clear effect of rooting depth on the acquisition of fertilizer N was observed, although monocultures of shallow rooting species performed poorly both in terms of yield and fertilizer N recovery. Mixing species that are temporally complementary in terms of nitrogen uptake, such as *L. perenne* in combination with *T. pratense* and/or *C. intybus*, allowed for increased fertilizer uptake and substantial biomass production at each period of regrowth.

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Modelling DM growth of multi-species grassland plots in the Netherlands

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Abstract

Natural and semi-natural grasslands show a great diversity of grass species and varieties, but the diversity is decreasing. Therefore it is of concern to preserve these areas. In the Netherlands farmers are subsidized to manage semi-natural grasslands in a proper way. A grass growth model was calibrated with a large set of data from experiments on intensively managed grasslands and semi-natural grasslands. This paper shows that the Dutch grass growth model as a component of the whole-farm model DairyWise is capable of predicting DM yield of semi-natural grasslands and the DM yield of intensively managed grasslands.

Keywords: DM yield, grass, grass growth, modelling, multi species, semi-natural grasslands.

Introduction

One of the objectives of the Multisward project is the development of tools to protect the diversity of grasslands all over Europe. In the Netherlands some semi-natural grasslands can be found, mainly in wet areas. Dutch farmers can get a so-called 'green subsidy' for extensively used grasslands. The yield of these grasslands (mainly used as grass silage from the first cut) will be used on dairy farms. To study the possibilities of using the yield on dairy farms, it is helpful to have information on grass production (in time, yield and quality). The Dutch whole-farm model DairyWise (Schils *et al.*, 2007) contains a grass growth model, based on results from intensively managed grasslands (mainly perennial ryegrass (*Lolium perenne*)) and some data from semi-natural grasslands with different species. The question is, whether it was permitted to use a mixed set of data for calibration and, subsequently, to use the model for intensively managed grasslands as well as for semi-natural grasslands. The aim of this study is to test whether the grass growth model is capable of predicting DM yield of semi-natural grasslands. We hypothesize that multiple species of grass in semi-natural grasslands will show a different growth curve than grass in intensively managed and highly productive grasslands.

Materials and methods

With a large set of data, the Dutch grass growth model was calibrated in two steps: first the N yield was predicted and secondly the conversion of N yield to DM yield was predicted. Based on the data, an empirical N-uptake and grass growth curve was made using regression analysis and REML for the conversion (Holshof, *in prep*.). Input variables were soil type, the N supply of the soil, the N application, start date of the cut (day number) and number of growing days. The majority of data (16238 records) were collected on intensively managed grasslands with 85-98% perennial ryegrass (*Lolium perenne*). However, a part of this calibration set consisted of data from experiments in the 1980s on semi-natural grasslands (2060 records) as collected and described by Korevaar (1986). On 11 locations, about every seven days a fresh plot was mowed during the growing period of a cut. This resulted in six harvests per plot per cut. On an annual base, four cuts were harvested. The plots differed in N application (0 kg N ha^{-1} for the first cut, 0, 40 or 80 kg N for the second and third cut and 0, 20 and 40 kg N for the last cut). There were four replicates. All plots were mown and the DM yield and the nutrient value of the grass were measured. The locations were mainly wet peat soils (from 1979 to 1982, six experiments). Furthermore there was poor sandy soil (1980-1982, three experiments) and river clay soil (1980, 1982, two experiments). Table 1 provides an overview of the locations and their botanical composition, divided in sub-classes according to De Vries *et al.* (1942).

Table 1. Overview of the experiments on semi-natural grasslands and their botanical composition (% good, moderate and inferior grasses and % herbs; De Vries *et al.*, 1942) used for calibration of the grass growth model.

Experiment	Year	Soil	Good	Moderate	Inferior	Herbs
1	1979	Peat	30	47	18	5
2	1979	Peat	17	41	28	14
3	1980	Peat	16	32	36	16
4	1981	Peat	45	7	34	14
5	1981	Peat	11	19	55	15
6	1982	Peat	11	33	34	22
7	1980	Sand	50	12	26	12
8	1981	Sand	61	2	29	8
9	1982	Sand	24	5	37	34
10	1980	river clay	34	41	12	13
11	1982	river clay	26	60	8	6
Average	Average		30	27	29	14

The 30% value for 'good species' in Table 1 consists mainly of perennial ryegrass (8%), rough-stalked meadowgrass (*Poa trivialis,*15%) and meadow fescue (*Festuca pratensis,* 3%). The moderate-value species (19%) are mainly creeping bent (*Agrostis stolonifera,*13%) and meadow foxtail (*Alopecurus pratensis*, 6%). The 28% of inferior grasses consist of velvet bent (*Agrostis canina*) and marsh foxtail (*Alopecurus geniculatis,* 14%). The herbs are mainly creeping buttercup (*Ranunculus repens*). The calibration of the prediction of the DM yield was done in several steps with a REML analysis. A factor 'grassland type' (semi-natural grasslands versus intensively managed grasslands) was added to the model and the significance of this factor on the DM yield was tested.

Results and discussion

The statistical analysis with REML showed no significant effect of the factor 'grassland type' on the prediction of the DM yield converted from the N yield ($P = 0.75$). Therefore, this factor was removed and the final model is made without this factor and used for predictions for both grassland types. In Figure 1, both the predicted DM yield by the calibrated model based on the total set of data and the measured DM yield are given. The dark dots represent the data of the semi-natural grasslands, and the light dots the data of the intensively managed grassland. The model slightly overestimates the DM yield above 6000 kg DM ha^{-1} cut^{-1}. Harvests on farms, however, are usually less than 4500 kg DM ha^{-1}. The N application of the semi-natural grasslands was between 0 and 200 kg N ha^{-1} yr^{-1}. Higher N applications could affect the botanical composition in favour of higher appreciated grass species. Figure 1 shows that the model is suitable for semi-natural grasslands in a range of low N input (unfertilized first cut and low fertilization after the first cut). This is in accordance with results of Cowling and Lockyer (1965). The difference between semi-natural grasslands and intensively managed grasslands is not the DM growth pattern, but the feeding value and the digestibility. Korevaar (1986) showed that the digestibility of the grass from semi-natural grasslands is about 15-20% lower than the grass from intensively managed grasslands.
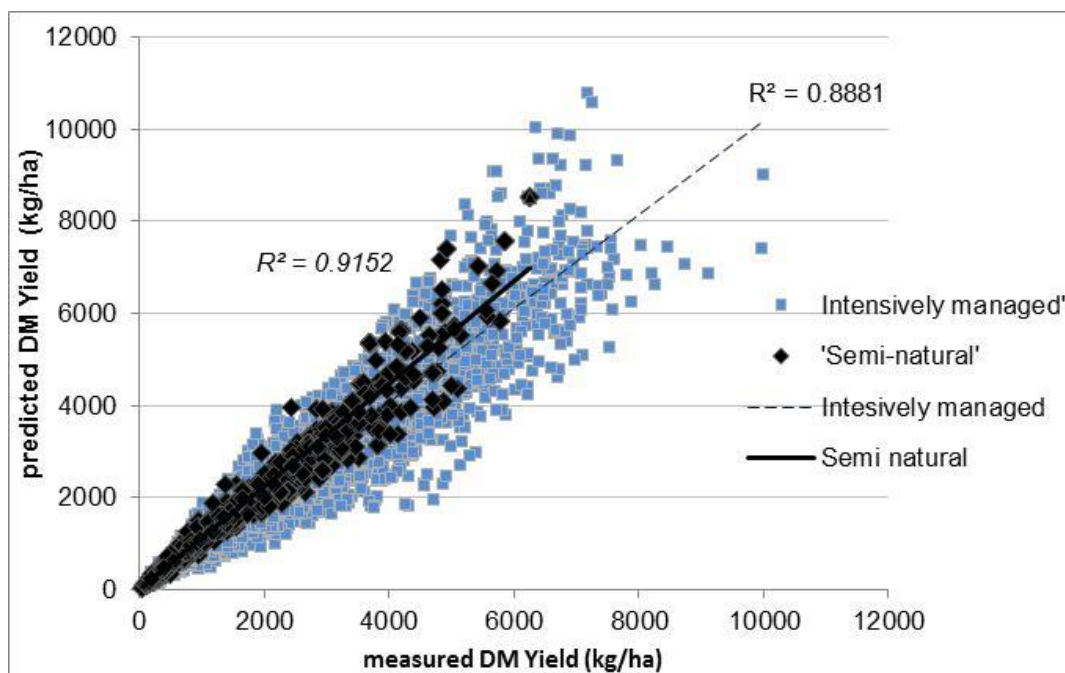
Figure 1. Measured and predicted DM yield for intensively managed and semi-natural grasslands.

Conclusions

We conclude that the Dutch grass growth model is capable of predicting the DM yield of semi-natural grasslands and also that of intensively managed grasslands. The prediction of the DM yield of semi-natural grasslands with this model, however, should be in a range of 0-200 kg N as annual application. The distinction between semi-natural grasslands and intensively managed grasslands could be based on a difference in N application and the digestibility of the grass rather than in a difference of the DM production.

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Interest of multi-species swards for pasture-based milk production systems

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Abstract

The objective of this 2-year study was to determine the potential of multi-species swards (MSS) for making secure forage availability in grazing dairy systems, and for increasing milk production on a per-ha basis. Four types of sward with increasing number of sward species (from pure perennial ryegrass to a mixture of five species including perennial ryegrass, two clovers, chicory and tall fescue) were compared with four block replicates. Treatments within blocks were simultaneously grazed by four homogeneous dairy herds at the same pasture allowance. Total grazing days per season or per year were unaffected by treatment, but milk output per ha was greater for grass-legume mixtures and MSS compared to pure perennial ryegrass swards. This was related to greater milk production per cow and per day. It is concluded that, under favourable weather conditions particularly in late spring and early summer, advantages of MSS on milk output on a per-ha basis are mainly due to an improved per-cow production rather than to increased pasture herbage production and grazing days/ ha.

Keywords: dairy system, forage mixture, milk production, grazing, chicory

Introduction

Multi-species swards (MSS) with legumes could form the basis of sustainable pasture-based milk production systems. Benefits of ryegrass-clover mixtures over ryegrass monocultures include: their potential to supply greater forage yields, the replacement of mineral N fertilizer inputs by symbiotic N_2 fixation by legumes, the reduction of total greenhouse gas emissions per kg of milk, the possibility of extending the pasture growth season, the greater nutritive value and the greater pasture DM intake and milk yield (Lüscher *et al.*, 2013). However, ryegrass-legume mixtures are not well adapted to dry and hot weather conditions. To prevent negative effects of summer drought, several cool-season species may be included in MSS. For instance, chicory is a deep-rooted forb with a high nutritive value and is well-adapted to dry summers (Barry, 1998). Tall fescue is a drought-resistant grass which can show higher annual forage yield than perennial ryegrass due to better tolerance of dry soil conditions. Nevertheless, the effects of MSS on dairy cow performance (Soder *et al.*, 2006) and milk production per ha at the system level are still relatively uncertain. The aim of this experiment was to compare four types of sown swards, differing in botanical composition, on seasonal and annual grazing days and milk production per ha when grazed by dairy cows.

Materials and methods

The experiment was carried out from September 2011 to August 2013, at the INRA farm of Méjusseaume (48.11° N 1.71° W; Le Rheu, France). The total grazing area (8.7 ha) was divided into 4 block replicates. Each block was divided into 4 paddocks randomly sown in Sept 2010 with either one of the four mixtures described in Table 1. It was hypothesized that the ability to produce forage in all seasons, together with improved tolerance of summer drought would increase with botanical complexity, i.e., from L to LTCF swards. Mineral nitrogen fertilization was similar between treatments (75 kg N/ha/year). Treatments were simultaneously grazed by four homogeneous herds of nine experimental autumn-calving Holstein-Friesian cows using a rotational strip-grazing system Pre-experimental reference periods were carried out at each season to randomize groups of cows. Grazing was organized by cycles and cows grazed non-experimental pastures as one single herd between 2 cycles. Within a grazing rotation, the four blocks were grazed successively with the two following management rules: 1) same grazing

calendar (i.e., same dates) between treatments to avoid time lag, and 2) similar pasture allowance (22 kg DM/cow/day > 3 cm) between treatments that define a medium to high grazing pressure. To combine both rules, extra non-experimental cows were needed to adjust the grazing pressure within block and between treatments according to differences in pre-grazing herbage mass. Cows grazed 20 h daily and received no feed supplements. Milk production was measured individually in two daily milkings. Pre-grazing pasture mass (> 3 cm), height (plate-meter), botanical composition (manual sorting) and chemical composition (oven drying) were determined before grazing at each block. Grazing days and milk output per ha were calculated considering the grazing calendar and both experimental and extra cows. Milk output per ha was calculated from the milk production of experimental cows and the grazing days per ha. Data were analysed by analysis of covariance. Orthogonal contrasts were used to test the effect of introducing legumes (contrast T: L *vs.* LT), the effect of MSS compared to single grass-legume mixtures (contrast M: LT *vs.* LTC/LTCF), and the effect of introducing tall fescue (contrast F: LTC *vs.* LTCF).

Table 1. Sowing rate (kg/ha) of each species and objectives of the four sward type treatments.

Treatment	No. of species	*Lolium perenne* Aberstar	*Trifolium repens* Alice	*Trifolium pratense* Segur	*Cicorium intybus* Puna 2	*Festuca arundinacea* Callina	Treatment interest
L	1	35	-	-	-	-	Control
LT	3	24	3	3	-	-	Legumes
LTC	4	22	3	3	1.5	-	Deep-rooting forb
LTCF	5	11	3	3	1.5	11	Drought-resistant grass

Results and discussion

Pastures were grazed during 13 rotations in 2 years, with an average of 2 cycles in autumn, 2.5 cycles in spring and 2 cycles in summer for each year. Weather conditions were globally good for pasture growth in both years, with rainy springs and medium temperatures in early summer, enabling the maintenance of pasture growth until end of July, including in L swards. On average, legumes represented 20% of DM in LT, LTC and LTCF; chicory represented 30% of DM in both LTC and LTCF; and tall fescue represented 10% in LTCF. All sward types were of good quality (Table 2). Pre-grazing pasture mass and number of grazing days per ha were unaffected by treatments, either at Year level (Table 2) or any season (Figure 1).

Table 2. Effect of MSS on pasture mass, sward chemical composition, ave cumulated grazing days/ha/year, average milk yield and milk output/ha/year of pastures grazed during two years.

Variable	Treatment[1]				RSD[2]	Contrasts[3]		
	L	LT	LTC	LTCF		T	M	F
Pasture mass (kg DM/ha > 3 cm)	2485	2706	2607	2583	976.6	ns	ns	ns
Pre-pasture height (cm, plate-meter)	12.6	13.7	15.8	15.2	4.23	ns	*	ns
Pasture CP (g/kg DM)	187	190	189	200	23.3	ns	ns	*
Pasture NDF (g/kg DM)	535	530	469	484	29.6	ns	***	ns
Pasture DM digestibility (g/kg)	752	734	776	758	2.5	ns	**	ns
Grazing days (/ha/year)	749	816	788	770	68.2	ns	ns	ns
Milk yield (kg/cow/day)	17.1	18.1	18.4	18.8	1.24	***	*	ns
Milk output per year (kg/ha/year)	14020	16123	15579	15568	1432.0	**	ns	ns

[1] See Table 1; [2] Residual standard deviation; [3] Sig.: *** ($P<0.001$); ** ($P<0.01$); * ($P<0.05$); ns, not significant.

This may be explained by the overall good pasture growth conditions which did not reduce the L growth rate, particularly in early summer. High levels of milk output per ha were reached in all treatments and high grazing pressure during the 2 years. Milk output per ha was greater

(P<0.01) on LT than on L, with no further increase between LT and the two MSS swards (Table 2). This increase between L and the 3 other treatments averaged 1737 kg milk/ha/year (+12%). The greater milk output/ha/year was mainly related to an increase in milk production per cow, which was greater (+1.0 kg/day, P<0.001) on LT than on L swards, as reported by Lüscher *et al.* (2013). A further increase in milk yield (+0.5 kg/day, P<0.05) was observed on MSS versus LT swards. This result can be explained by a slightly greater digestibility of pasture herbage (Table 2) and probably also to a greater DM intake considering the low NDF concentration and high voluntary intake previously reported for chicory (Barry, 1998).
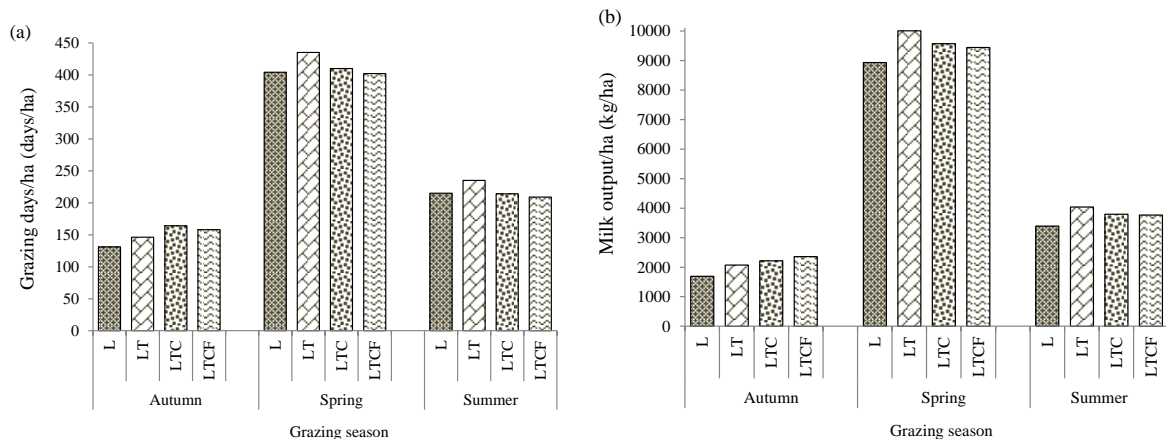
Figure 1. Effect of MSS on (a) grazing days/ha per season and (b) milk output/ha per season and per year. Grazing season distribution: autumn (from mid-September to mid-December), spring (from mid-March to mid-June) and summer (from mid-June to mid-September).

Conclusion

Advantages of MSS on dairy cow production on a-per ha basis are mainly due to an improved per-cow production rather than to increased pasture production or grazing days per ha.

Acknowledgements

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Influence of ryegrass alone or blended with clover and chicory on feed intake and growth performance of steers

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Abstract

The purpose of the experiment was to compare the influence of a sward of four species (FS) with that of a perennial ryegrass monoculture (RG) on feed intake and growth of fattening steers. The FS sward was sown with a mixture of 50% perennial ryegrass, and 50% chicory, white clover and red clover (in a ratio of one-third each); in the case of both the FS and the RG sward, half was used for cutting (C) and half as grazed pasture (P). Over 8 cycles, each lasting 2 weeks, two groups, each consisting of 3 Angus (AN) and 3 Limousin (LM) steers, ingested either the FS mixture or the RG, and then the opposite, in turn, with a change of system (C and P) every other cycle. The intake, weighed in the stable (C) and estimated at pasture using the n-alkane double-indicator technique, was significantly higher for the FS forage in the case of both C and P (*P*=0.022). In contrast, the daily growth was highest with the RG forage (*P*=0.036), pointing to better feed conversion efficiency, expressed in kg DM intake per kg average daily gain (ADG). A lower gut content and consequently a lower body weight at the end of the 14-day periods of FS intake is the likely explanation for these unexpected results because the lower NDF content of the FS forage probably increased its transit rate.

Keywords: multispecies, sward, pasture, cutting, beef steers, intake, growth

Introduction

Grassland systems can make a vital contribution to meeting the various challenges facing agriculture today, which include limiting the impact on the environment, preserving or increasing biodiversity, supporting sustainable development and preserving landscapes while continuing to produce high-quality food. Against this background, the use of forage areas in the form of grazed pasture responds to ethical needs (consumer wishes, closeness to nature), technical requirements (absence or restriction of the use of commercial nitrogen fertilizers) and economic needs (Peyraud and Baumont, 2002; Roinsard, 2011). Similarly, the plant species composition of swards may constitute an increasingly important lever in the future, particularly in terms of safeguarding forage systems against climate change. The importance of the complementarity of different species has been noted in many reports that have stressed, for example, the benefits of different root systems (Fustec *et al*., 2010), the improvement in nutritional value (Rodriguez *et al*., 2007; Niderkorn *et al*., 2008) and the better ingestibility (Roinsard, 2011), as well as the more stable yield (Fustec *et al.*, 2008). Among the animals with the potential to optimize the use of these pastures, fattening cattle offer the greatest intake potential after dairy cows. Differences between breeds may be found, as illustrated by Dufey *et al*. (2002) in a comparison of 6 beef breeds. The objective of this experiment, carried out in the framework of the EU Multisward project (www.multisward.eu), was to compare two different swards (multi-species and monoculture), using two systems (pasture and stable) and steers of two different breeds (Angus and Limousin), in terms of growth performance.

Materials and methods

The experiment was performed on the experimental farm of Agroscope at Posieux (650 m altitude) from 27 April to 17 August 2012. A mixture of four species (treatment FS) comprising perennial ryegrass (*Lolium perenne*) cv. Alligator, white and red clovers (*Trifolium repens* and *pratense*) cv. Hebe and Dafila, and chicory (*Cichorium intybus*) cv. Puna II, was sown in August

2011 on a plot of 2 ha. The quantities sown were 17.5, 2.67, 2.74 and 1.09 kg/ha respectively, corresponding to ½, 1/6, 1/6 and 1/6 respectively of the usual quantity sown to grow a monoculture of the individual species. In parallel, a monoculture of perennial ryegrass (*Lolium perenne*) cv. Alligator (treatment RG) was sown on an adjacent plot of 2 ha at the rate of 35 kg/ha. Half of the 2 ha of each plot was intended for cutting and the other half as grazed pasture. To ensure that the sward established well, the first application of mineral fertilizer in the spring of the year of the experiment was doubled for the RG treatment to 54 kg N ha^{-1}, compared to 27 kg for the FS treatment. This had been preceded by an application of 40 kg N in the form of organic fertilizer. Subsequently, during the period of the experiment, a total of 81 kg N was applied in three applications of 27 kg each to both of the swards, giving a total for the year of 148 kg N for the FS treatment and 175 kg N for the RG treatment.

Six Angus (AN) and six Limousin (LM) steers produced from suckler cows, aged 14.2 months and weighing 447±33 kg, were divided into two groups each consisting of 3 AN and 3 LM cattle. During a total of eight consecutive two-week cycles, the two groups were kept either in a stable or at pasture, with a change of system every other cycle. One of the groups received the RG treatment and the other the FS treatment during the first cycle of a system; then the treatments were reversed for the second cycle. During the periods in the stable, forage was provided *ad libitum* and the daily forage intake was measured individually using feed containers mounted on electronic weighing machines (Insentec B.V., Marknesse, The Netherlands). The first 3 days of each cycle served as an adaptation period in both systems. The results of intake in the stable are based on the last 11 days of a cycle. In parallel, as well as during grazing periods, the intake of forage was estimated using the n-alkane double indicator technique. In each cycle, n-alkane HC32 was dosed orally in a gelatine capsule twice-daily during 11 days. Faeces were collected by rectal sampling twice-daily, from day 8 to day 11. During grazing, the same DM quantity of forage was offered each day to the two groups of animals. This quantity was calculated on the basis of biomass quantity measured each week on the test plots, taking account of the grass growth measured with an electronic rising plate meter (Jenquip, Feilding, New Zealand). During the first two and the last two cycles of grazing, the quantity offered was equal to 94% and 96%, respectively, of the average intake measured in the stable during the previous cycle. The animals were weighed before the start of each new cycle and at the end of the last cycle. The data were analysed using a General Linear Model (NCSS 2007, Dr. Jerry L. Hintze, Kaysville, Utah).

Results and discussion

Intake in the stable *(ad libitum)* was higher in the FS treatment than in the RG treatment (9.23±0.85 compared to 8.84±0.82 kg DM per animal per day) for an average of 4 cycles (*P*=0.002). The correlation with intake estimated by the HC31-HC32 n-alkane marker pair was more reliable than with the HC33-HC32 marker pair (R^2=0.71 compared to 0.59). The analysis of intake over the whole experimental period (4 cycles in the stable and 4 cycles at pasture) was therefore based on the estimate obtained with the HC31-HC32 marker pair. With a difference of 0.4 kg of DM in favour of FS compared to the RG treatment, the significant effect measured in the stable was confirmed (8.28±1.70 compared to 7.88±1.55; *P*=0.022). These results support the findings of Baumont *et al.* (2008), Ginane *et al.* (2008) and Roinsard (2011), who observed an associative effect between different species such as grasses and legumes, which was positive in terms of ingestibility. In the stable the trend was for the DM intake of the LM cattle to be 7% (0.64 kg) lower than in the case of the AN (*P*=0.095). This difference was less marked over all the cycles combined, with an average intake of 7.91±1.45 kg DM d^{-1} for the LM breed compared to 8.26±1.78 for the AN breed. This trend is in agreement with the results of Dufey *et al.* (2002), who observed an 8% lower intake for LM cattle compared to AN cattle (n.s.), and with results published by Faverdin *et al.* (1997), who concluded that the LM breed has a 10% lower intake

capacity than the other breeds. With a gap of nearly 320 g ADG in favour of RG, there was a statistically significant difference between sward treatments in the stable cycles (1535±526 g/d for FS compared to 1852±594 g/d for RG; P=0.003). This disadvantage of the FS sward type was confirmed when results were averaged over the eight experiment cycles (stable and pasture), with a reduction in ADG of 21% compared with the RG sward (746±1094 g/d for FS compared to 943±1164 g/d for RG; P=0.036). No significant effect of cattle breed was observed. This lower animal growth for the FS treatment in spite of a higher intake indicates a much less-efficient feed conversion efficiency (6.94 kg DM per kg ADG for the FS treatment, cf. 5.29 kg DM per kg ADG for the RG treatment; P=0.001). A lower gut content and consequently a lower body weight at the end of the 14-day periods of FS intake is the most likely explanation for these unexpected results because the lower NDF content of the FS forage probably increased its gut transit rate.

Conclusion

Supplying a mixture of four forage species to cattle both in the stable and at pasture resulted in increased intake compared with a monoculture of perennial ryegrass. The results of daily weight gain and feed efficiency showed an apparent negative effect of the four-species mixture which could not be explained conclusively. Feeding trials without changing the type of forage would elucidate this issue and help to optimize the composition of the mixture with a view to improving the efficiency of forage utilization in cattle.

Acknowledgement

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Associative effects between forage species on intake and digestive efficiency in sheep

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Abstract

There is evidence that multi-species swards can increase biomass productivity and provide a number of ecosystem benefits. However, little is known regarding the possible interactions between forage species that can modulate positively or negatively the use of nutrients by ruminants. The objective of this study was to provide a better understanding of the associative effects between some forage species on intake and digestion parameters. Three sheep-feeding experiments were conducted according to a repeated Latin square design using models of simple forage mixtures under the form of fresh forage or silage, and during which, intake behaviour, DM digestibility and methane emissions were measured. Synergies between cocksfoot silage and red clover silage, and between ryegrass and chicory, were observed on DM intake and eating rate, with optima for the proportion 50:50. For the cocksfoot-red clover association, the synergy was also observed on daily intake of the digestible fraction that can reflect animal performances. No associative effect was observed on methane yield and the lowest emissions were observed for pure red clover and pure chicory.

Keywords: associative effects, grass-legume mixtures, chicory, intake, digestion, methane

Introduction

Diverse pastures are considered as having the potential to better serve production and ecosystem services than species-poor grasslands. However, there is a need for an improved understanding of the utilization of complex grasslands by ruminants to optimize their management. This implies investigations of animal responses to multi-species swards and, in particular, a better understanding of the interactions that can occur between plants on digestion, intake and pollutant emissions as enteric methane. Indeed, the digestibility and feed intake of a combination of forages can differ from the balanced median values calculated from forages considered separately leading to synergistic or antagonistic effects instead of simple additivity (Niderkorn and Baumont, 2009). The objective of this study was to assess the associative effects between some common or less known (e.g. chicory) forage species on intake and digestive processes in sheep.

Materials and methods

Three sheep-feeding experiments were conducted at INRA Clermont-Ferrand-Theix (France) between 2010 and 2012. Models of simple forage mixtures were designed under the form of fresh forage or silage according common use for the tested species. The combinations tested were i) binary mixtures of silages of cocksfoot (*Dactylis glomerata* L., cv. Starly) and red clover (*Trifolium pratense*, cv. Diadem) in five controlled proportions (in % dry matter (DM), 100:0; 75:25; 50:50; 25:75; 0:100); ii) binary mixtures of fresh forages of perennial ryegrass (*Lolium perenne* L., cv. AberAvon) and white clover (*Trifolium repens*, cv. Merwi) in the same proportions; and iii) mixtures of fresh forages containing chicory (*Cichorium intybus*, cv. Puna II): 100% ryegrass, 50% ryegrass + 50% chicory, 100% chicory, 50% ryegrass + 25% white clover + 25% chicory. For each experiment, housed rumen-cannulated sheep were used in a repeated Latin-square design, 4×4 or 5×5 according to the number of treatments. Mixtures were prepared from five-weeks regrowth plants cultivated in pure swards. Each experimental period consisted of an adaptation week to diet followed by a measurement week. During the

measurement period, chemical composition of plants, intake kinetics and behaviour (Baumont *et al.*, 2004), DM digestibility, and methane emissions using the SF_6 tracer method according to the procedure described by Martin *et al.* (2008) were determined. Data were analysed using the MIXED procedure of SAS® v.9.1 software. Linear and quadratic contrasts were tested to highlight potential associative effects between species.

Results and discussion
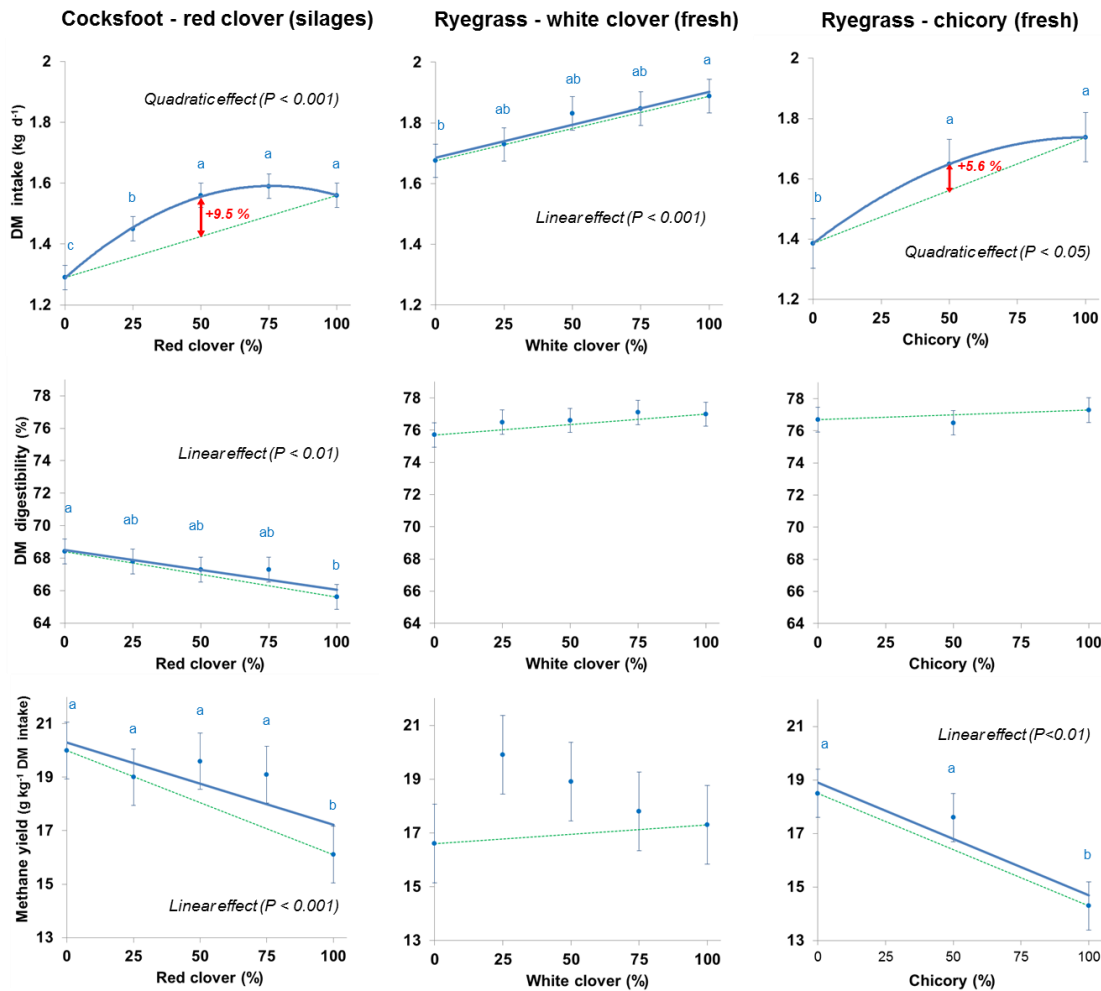
Figure 1. Voluntary dry matter (DM) intake, DM digestibility and methane yield in sheep fed with different proportions of cocksfoot and red clover silages, ryegrass and white clover (fresh), and ryegrass and chicory (fresh). Full lines represent smoothed linear or quadratic responses, and dotted lines represent theoretical responses calculated from values obtained from pure forages.

Significant positive quadratic effects were observed between silage of cocksfoot and silage of red clover ($P < 0.001$), and between fresh ryegrass and fresh chicory ($P < 0.05$) on voluntary DM intake indicating synergistic effects (Figure 1). The optimums were observed with the proportions 50-50, and the differences between the values measured for the plant combinations and the balanced median values from pure forages were +9.5% and +5.6% in voluntary DM intake for the mixtures cocksfoot-red clover and ryegrass-chicory, respectively. Adding a third species (chicory) did not improve intake and digestive efficiency of the ryegrass-white clover mixture (data not shown).

These synergies did not seem to be due to a more efficient digestion, as positive quadratic effects were not observed on DM digestibility ($P > 0.05$). For the mixture cocksfoot-red clover, a quadratic effect was observed on daily eating rate ($P = 0.008$) suggesting a greater motivation to eat. For all the mixtures, very strong positive quadratic effects ($P < 0.001$) were observed on DM intake and eating rates during the main meals distributed in the morning and the afternoon,

indicating that the diversity in the ration stimulated intake in the short-term. A particularly relevant result was observed with the mixture cocksfoot-red clover as a synergy was also observed on daily intake of the digestible fraction that can be seen as an indicator of animal performances (Niderkorn *et al.*, 2012). No associative effect was observed on methane yield (emissions in g/kg DM intake, $P > 0.05$). The lowest emissions were observed for pure red clover and pure chicory.

Conclusion

Taken together, our results indicate that synergy between some species in binary mixtures can be observed on voluntary intake in sheep, with an optimum for the proportion 50:50 without associative effects on methane emissions. Synergy seems to be rather due to a greater motivation to eat than to a more efficient digestion.

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Effects of restricting access time to pasture on late lactation dairy cow production

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Abstract

The objective of this study was to examine the effect of restricted access to pasture in the autumn on the milk production, grazing behaviour and dry matter intake of late-lactation spring-calving dairy cows. Forty-eight cows were assigned to one of four treatments: full-time access to pasture (22H); two 5-hour periods of access to pasture after a.m. and p.m. milking (2×5H); two 3-hour periods of access to pasture after a.m. and p.m. milking (2×3H;) and alternating between full time and 2x3H access to pasture (2x3HV). Dry matter intake, measured during week 3, was similar for all treatments (15.1 kg/cow/day); consequently there were no differences in milk or milk solids yield (13.2 and 1.15 kg/cow/day, respectively) between treatments. This was due to changes in the cows grazing behaviour as, when access time to pasture was restricted, the grazing intensity increased leading to higher intake per minute and per bite. This indicates that restricted access time to pasture can be implemented on dairy farms in autumn with no reduction in dairy cow production.

Keywords: restricted access, grazing, late lactation, dairy cows

Introduction

Including grazed herbage in the diet of lactating dairy cows in late autumn/winter maintains milk protein output due to the higher energy and crude protein concentrations of grass during this period compared with that of grass silage (Dillon *et al*., 1998). There is scope to increase the length of the grazing season on Irish dairy farms as currently only 241 grazing days are being achieved (Creighton *et al*., 2011). Restricted access time to pasture can be used as a tool to increase the length of the grazing season. In spring, restricted access time to pasture has been shown to have no effect on dairy cow production (Kennedy *et al*., 2009), whereas Pérez-Ramírez *et al*. (2008) reported that restricting access time to pasture reduced milk yield and composition during the spring/early summer. Studies reporting the effects of restricted access to pasture during the autumn are limited. The objective was to examine the effect of restricted access to pasture in autumn on the milk production, grazing behaviour and dry matter intake (DMI) of late-lactation spring-calving dairy cows.

Materials and methods

Forty-eight (12 primiparous and 36 pluriparous) Holstein-Friesian dairy cows (mean calving date – 11 March, s.d. 23.1 days) were balanced on parity (2.6, s.d. 1.20), milk yield (13.3, s.d. 2.15 kg), body weight (501, s.d. 60.0 kg), milk fat (45.1, s.d. 4.61 g/kg), protein (36.7, s.d. 2.80 g/kg) and lactose concentrations (43.3, s.d. 1.41g/kg) in a randomized block design. From 26 September to 4 November 2012 cows were randomly assigned to one of four treatments: 22 hours (full time) access to pasture (22H; control); two 5-hour periods of access to pasture after a.m. and p.m. milking (2×5H); two 3-hour periods of access to pasture after a.m. and p.m. milking (2×3H); and alternating between full time and 2x3H access to pasture, with no more than 3 continuous days on any one regime, e.g. Monday – full-time access, Tuesday - 2x3H access, Wednesday - 2x3H access; Thursday – full-time access *etc*. (2×3HV). All treatments were offered fresh herbage daily at a daily herbage allowance of 17 kg DM/cow/day (>4 cm); no additional supplementation was offered. Treatment groups grazed as separate herds adjacent

to one another. Pre-and post-grazing sward heights were measured daily. Milk yield was recorded daily and milk composition, body condition score (BCS) and bodyweight (BW) were measured weekly. Dry matter intake was measured during the third week of the study using the n-alkane technique. Grazing behaviour was recorded using IGER grazing recorders. Grazing behaviour, DMI, milk yield and composition were analysed using covariate analysis in SAS, terms for parity, treatment, experimental week and the treatment×week interaction were included in the model.

Results and discussion

The pre-grazing herbage mass of swards offered to all treatments was similar (1544 kg DM/ha). The 2×3H had a significantly higher post-grazing sward height (4.6 cm; *P*<0.001) than all other treatments (4.2 cm). Similar to previous experiments (Kennedy *et al.*, 2009) that have investigated the effects of restricted access to pasture, the lack of differences in cow production was due to the change in the cows grazing behaviour. Although cows in the 2x3H and 2x5H had a shorter grazing time they increased their intake per minute and per bite compared to the 22H cows (Table 1) to compensate for their restricted access to pasture. Consequently, there were no differences in grass DMI (15.1 kg/cow/day) or milk yield (13.2 kg/day), milk fat (48.2 g/kg), protein (39.0 g/kg) or lactose content (42.6 g/kg) or milk solids yield (1.15 kg/day). Similarly, there was no effect of treatment on end BW (483 kg) or BCS (2.66). Interestingly, when the 2x3HV were given full-time access to pasture their grazing behaviour was similar to the 22H cows, and when their pasture access time was restricted to two 3-hour periods they behaved like the 2x3H cows.

Table 1. Effect of restricting pasture access time of late-lactation dairy cows on grass dry matter intake (GDMI), milk production and grazing behaviour.

	22H	2×3HV	2×5H	2×3H	S.E.D	Sig
GDMI (kg/day)	15.5	15.1	15.0	14.9	0.53	0.850
Milk yield (kg day)	13.2	13.3	13.7	12.6	0.55	0.197
MSY (kg day)	1.17	1.14	1.18	1.09	0.051	0.238
Grazing time (min/day)	565[a]	460[b]	487[b]	358[c]	32.7	0.001
GDMI/min (g)	27.6[a]	33.9[b]	30.8[a]	41.3[c]	2.66	0.001
GDMI/bite (g)	0.49[a]	0.54[a]	0.50[a]	0.68[b]	0.051	0.001

22H = 22 h access to pasture; 2 × 3 HV = Alternating between full time and two 3 h periods of access to pasture, with no more than 3 continuous days on any one regime; 2 × 5 H = Two 5 h periods of access to pasture; 2 × 3 H = Two 3 h periods of access time to pasture.

Conclusion

Late-lactation dairy cows can increase their intake per minute and intake per bite when access to pasture is restricted, resulting in no differences in grass DMI or milk production. There was no effect of alternating access time between 22H and 2×3H on milk production and DMI in the 2×3HV treatment. Therefore, it would be possible for restricted access time to pasture to be implemented on dairy farms in autumn with no reduction in dairy cow production.

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Theme 5 special paper

Grassland term definitions and classifications adapted to the diversity of European grassland-based systems

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Abstract

Grasslands are represented in an unsatisfactory manner in agricultural statistics. The official definition of grasslands does not include vast areas of grazed shrubby and wooded ecosystems. Temporary grasslands are recorded as 'Leguminous plants' and 'Temporary grass' which induces doubt on the classification of grass-legume mixtures and often leads to the underestimation of temporary grassland areas. Some terms like 'meadows' and 'pastures' are often used in an imprecise and misleading way. The term 'rough grazing' does not include all types of natural and semi-natural grasslands, especially all types of rangelands, forest pastures and traditional hay meadows. It can thus not represent all species-rich grassland types. Improvements of the current situation are proposed. They consist mainly in: (i) better definitions of grassland terms including for pastures and meadows, permanent, agriculturally-improved, semi-natural and natural grasslands; (ii) the classification of temporary grasslands in three categories: pure legume sowings, pure grass sowings and grass-legume mixtures; (iii) the classification of permanent grasslands in three categories: agriculturally-improved, natural and semi-natural, no longer used for production; and (iv) the introduction of a new category for grazed fallow land. The paper presents a comprehensive classification of fodder and grassland types in the agricultural area and a multilingual vocabulary.

Keywords: Grassland term definition, agricultural statistical classification

Introduction

A Working Group on 'Grassland Term Definition' was set up during the 24th General Meeting of the European Grassland Federation (EGF) that took place on 3-7 June 2012 in Lublin (Poland). It gathered together 22 experts from 13 countries (Belgium, Bulgaria, France, Germany, Italy, Poland, Romania, Slovakia, Spain, Sweden, Switzerland, The Netherlands, United Kingdom). The group is thus representative of the diversity of thinking of European grassland researchers.

The purpose of the creation of the Working Group was to support the European Union (EU) Institutions to enable better account to be taken of all the diversity of grasslands into the Common Agricultural Policy (CAP). The EGF adopted a resolution on the reform of the CAP during its Business Meeting. This resolution was sent to a wide group of decision makers of the EU. The participants at the EGF conference decided that a glossary would also be useful in addition to their proposals on grassland in the future CAP. It was thus decided to draft a vocabulary of grassland terms.

After contacts with Eurostat, it appeared that this European Commission organization would also be interested in a better definition and classification of grassland terms. This classification could improve the present system of data collection and could lead to a better consideration of the importance and diversity of grasslands in European agricultural statistics. The system should be simple but at the same time be able to collect some new agri-environmental indicators on grasslands and grassland-based systems. This could be the basis of a better recognition of ecosystem goods and services that grasslands can provide. The present text is a trade-off

between the level of precision that is necessary to reach the objectives described above and the practical aspects related to data collection, in particular from farmers.

The present text is largely inspired by the work of Allen *et al.* (2011) who defined many grassland terms at a global level. In this work, the Working Group adapted these definitions to European specificities. The text is restricted to agricultural grasslands; other types like recreational (e.g. lawns of sport fields) and ornamental grasslands are not considered.

Grassland term definitions

1. **Fodder areas**: Part of the agricultural area that includes permanent grasslands, arable fodder crops and grazed fallow lands.

2. **Arable fodder crops**: Annual, biennial or perennial species sown on arable land for the production of forage and harvested as green material. They include temporary grasslands, green cereals (C3 species such as oats, barley, spelt, triticale, rye and C4 species such as maize and sorghum), green cereal-other crops mixtures, fodder roots, some *Brassicaceae* and *Compositeae* (e.g. sunflower) species.

   Additional remarks:

   Crops that are harvested as grain (cereal grain and pulses) and used for animal feeding are not classified in fodder crops.
   Cereals can represent a resource for mixed farming systems (livestock, grasslands and grain cereal production). This is traditionally the case in Mediterranean areas. Even when sown for grain production, their management can be flexible according to weather conditions prevailing during the growing season. For instance, cereal crops for grain production can be grazed in winter and then harvested for grain production, or grazed only when the predicted grain production will not cover the costs of mechanical harvesting.
   If cereals are harvested green, by grazing or harvested for silage as immature cereals, they should be defined as fodder crops. C3 cereals such as oats, barley and wheat can sometimes be mixed with other crop types like annual legumes (e.g. pea, vetch) and harvested green. These mixtures should also be considered as fodder crops.

3. **Grasslands**: Land devoted to the production of forage for harvest by grazing/browsing, cutting, or both, or used for other agricultural purposes such as renewable energy production. The vegetation can include grasses, grass-like plants, legumes and other forbs. Woody species may also be present. Grasslands can be temporary or permanent.
   Two management categories can be identified:

   - **Meadows**: grasslands that have been harvested predominantly by mowing over the last 5 years[1] or since the establishment of the sward if it is less than 5 years old.
   - **Pastures**: grasslands that have been harvested predominantly by grazing over the last 5 years[2] or since the establishment of the sward if it is less than 5 years old.

4. **Permanent grasslands:** Grasslands used to grow grasses or other forage (self-seeded or sown and/or reseeded) and that have not been completely renewed after destruction by ploughing or spraying (herbicide) for ten years or longer. They can be agriculturally improved, semi-natural, natural or no longer used for production.
   European permanent grasslands can be dominated by:

   - one or several grass species;
   - one or several grass species and one or several legume species;

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[1] Where there has been a recent change in the management strategy (more recently than 5 years), the new management type has to be taken into account.

- grasses, several forb species and possibly legume species;
- grass-like species and possibly forb species;
- shrubby zones (see 'semi-natural grasslands' for more information);
- grazed wooded areas (see 'semi-natural grasslands' for more information).

Additional remarks:

Long-term grasslands provide more ecosystem goods and services than short-term grasslands (e.g. carbon storage, biodiversity levels). In a previously cultivated soil a minimum duration of ten years is necessary in most situations to approach a level of soil organic carbon that is representative of long-term permanent grasslands. A period of ten years is also considered as a minimum for reaching soil biodiversity, and especially higher plant diversity, which is characteristic of long-term permanent grasslands for a given intensification level.

The effects of cultivating and reseeding, however, can vary according to the region and type of grassland, and the acceptable frequency of cultivation also varies. For example, in Mediterranean areas, self-seeded permanent grasslands consisting mainly of annuals can be tilled (light harrowing, not deep ploughing) every few years without destroying floral biodiversity. Harrowing is a quite common practice to control scrub invasion, e.g. in Dehesas/Montados. In these conditions, permanent grasslands can be tilled more frequently than once every ten years.

5. **Natural and semi-natural grasslands**: Low-yielding permanent grasslands, dominated by indigenous, naturally occurring grass communities, other herbaceous species and, in some cases, shrubs and/or trees. These mown and/or grazed ecosystems have not been substantially modified by fertilization, liming, drainage, soil cultivation, herbicide use, introduction of exotic species and (over-)sowing. The occurrence of natural grasslands is not related to human activities, in contrast to the latter.

Additional remarks:

Occasional liming on acidic grasslands, or the application of very low amounts of organic fertilizers, if not combined with other 'improvement' techniques, are not considered to modify habitats substantially. If not associated with higher fertilization or stocking rate, drainage can transform wet semi-natural grassland into mesophilous semi-natural grassland. Although most semi-natural communities give low production, some of them, such as purple moor grass (*Molinia caerulea*) or tall sedge (*Carex* spp.) communities, can be quite productive.

Natural vegetation types are communities where the vegetative cover is in dynamic balance with the abiotic and biotic (human species excluded) forces of its ecosystem. Semi-natural vegetation is not planted/sown by humans but is influenced by human actions such as grazing, cutting or burning. Previously cultivated areas that have been abandoned and where vegetation is regenerating may also evolve to semi-natural vegetation. In contrast with natural vegetation, semi-natural communities thus need regular anthropogenic disturbances to be maintained.

Semi-natural grasslands are usually biodiverse. They include[2]:

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[2] These communities correspond for instance to the 1430, 21A0, 4010-4040, 5130, 52, some 53, 6210-6270, 62A0-62D0, 6310, 6410-6460, 6510-6530, 9070 NATURA 2000 Codes of Annex I of the Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, the so-called 'Habitats Directive'. They include also non NATURA 2000 habitats such as the *Cynosurion*, *Bromion racemosi* and *Alopecurion*.

- grazed (pastures) or mown (meadows) grasslands in the plain or low mountain areas including wet areas (riparian vegetation, valleys, flood areas) where grazing and mowing are usually combined in time and/or space;
- montane and sub-Alpine meadows and pastures;
- grazed steppes and dry pastures;
- land crossed during transhumance where the animals spend a part of the year (approximately 100 days) without returning to the holding in the evening;
- grazed wooded areas (agroforestry areas, Dehesa and Montado type for example). Forestland that produces, at least periodically, spontaneous native understorey vegetation that is grazed and where shrubs and trees are browsed is also considered as grazed semi-natural vegetation, including fire-break lines;
- grazed/browsed shrubby zones (e.g. heath, maquis, matorral, garrigue).

Natural grasslands are also often biodiverse; they cover limited areas in Europe. They include for example[3]:

- Alpine and boreal tundra grasslands (beyond the tree line);
- rupicolous pannonic grasslands for instance of Hungary;
- Macaronesian mesophile grasslands from the Atlantic islands (e.g. Azores);
- steppic grasslands for instance of Romania, Russia and Ukraine;
- Mediterranean xeric grasslands (e.g. main Mediterranean islands and *Stipa* grasslands in SE Spain);
- Grasslands developed on saline soils.

'Agroforestry' is the integration of woody perennials, crops and/or grasslands on an area of land. Trees may be single or in groups, inside parcels (silvoarable agroforestry, silvopastoralism, grazed or intercropped orchards) or on the boundaries (hedges, tree lines). Silvoarable systems are extensively used in Mediterranean areas; they include fodder crop rotation under the trees for feeding animals during shortage periods. Agroforestry systems are obtained by planting trees on agricultural land or by introducing agriculture in existing woodland (e.g. silvopasture).

A silvopastoral system, like the Dehesa/Montado, has the chief aim of providing food for livestock while taking advantage of the presence of trees (for example for shade, shelter, milder microclimate, 'nutrient pump', strategic browsing and acorn grazing), whilst obtaining a secondary profit from trees in the mid/long term (from, for example, cork, wood, firewood).

Silvopastoral systems also include those areas where the understorey of a forest is grazed. It combines grazing with tree production (wood, fruits, fodder) and the maintenance of the forest ecosystem. This system reduces fire risk by controlling inflammable understorey and preserves biodiversity through animal disturbance. In some high forest, the main productive use is wood, but many grazed forests have minimal value for timber or firewood, and grazing is really the most important productive use (e.g. in Mediterranean areas). There are different situations and the official classifications and statistics should recognize this.

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[3] Annex I of the Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, the so-called 'Habitats Directive' recognizes 9 habitat types of natural grasslands (8 of them can be grazed: 6110, 6120, 6140-6190 NATURA 2000 Codes). The following habitats should also be considered as natural grazed communities: 1330, 1340, 1410-1430, 1630, 2130-2150, 21A0, 2230, 2240, 2330, 2340, 4060, 9050.

6. **Agriculturally improved permanent grasslands**: Permanent grasslands on good or medium quality soils, used with more frequent defoliations, higher fertilization rates, higher stocking rates and producing higher yields than natural and semi-natural grasslands.

Additional remarks:

Agriculturally improved permanent grasslands can be dominated by:

- one or several grass species;
- one or several grass species and one or several legume species;
- grasses, one or several forb species and possibly legume species.

Agriculturally improved permanent grasslands are often classified, in terms of production, on the basis of the proportions of high-, medium- and low-productivity/quality grasses as well as on the proportion of legumes.

7. **Permanent grasslands no longer used for production**: Areas of permanent grasslands, regardless of the grassland type and the previous use, upon which the produced biomass is no longer used for agricultural production purposes, but which are maintained in good agricultural and environmental condition by appropriate measures.

8. **Temporary grasslands**: Grasslands sown with forage species that can be annual, biennial or perennial. They are sown on arable land and can be integrated in crop rotations or sown after another grassland vegetation. They are kept for a short period of time, from a couple of months to (usually) a few years. They can be established with pure sowings of legumes, pure sowings of grasses or grass-legume mixtures.

Additional remarks:

This category includes 'Leguminous plants' that are pure stands of leguminous plants or mixtures of predominantly leguminous plants mixed with grasses.

Temporary grasslands can be grazed or harvested green as hay or silage.

9. **Rangelands**: Extensive, large-scale grazed grasslands. Rangelands can be fenced or not but they are usually not fenced, so a shepherd is often needed.

Additional remark:

Rangelands are dominated by grazed semi-natural vegetation. They may include natural and semi-natural grasslands, shrublands, steppes, tundras, alpine communities, marshes and the understorey of forestland.

10. **Grazed fallow lands**: Extensively grazed uncultivated land after a cropping episode. The duration of the fallow is typically between one and four years. The land is then cropped again.

Additional remark:

Fallow lands are very commonly grazed in Mediterranean areas for livestock feeding. They are also important for wildlife (e.g. breeding birds) and soil conservation.

11. **Grazed common lands**: Permanent grasslands where two or more persons have the right to let their animals grazing concurrently; in some cases these rights are not permanently vested in the same individuals but are allocated from time to time by a body with legal authority to do so.

Additional remarks:

Common lands are part of the utilized agricultural area. They can be private or public (state, parish, *etc*.). They are generally semi-natural, but not always; some common lands have been 'improved' by reseeding and fertilization.

Rangeland is mostly common land, but not always; it can be in sole use[4]. Most common land is rangeland, but not always; it can consist of grassland, forest, horticultural or other land.

Classification of grassland types into an agricultural statistics system

Preamble

In the classification system described below, three main ideas are introduced:

- Permanent grasslands are described in three main categories:
  - Agriculturally improved permanent grasslands
  - Natural and semi-natural grasslands
  - Permanent grasslands no longer used for production
- The existing Eurostat category 'Fodder crops/Leguminous plants' has been amalgamated with the category 'Fodder crops/Temporary grass' so creating a new category 'Fodder crops/Temporary grasslands'
- A new category is introduced for 'Grazed fallow land'.

Almost no surface data were collected in the past in Europe for the area of 'Natural and semi-natural grasslands'. They differ from 'Agriculturally improved permanent grasslands' since they are usually richer in biodiversity because of a lower intensification rate and less modification of their habitats. Statistical data on these natural and semi-natural grassland types could thus be an important biodiversity indicator. Two main types can be defined: 'Pastures' (including rangelands, rough grazing, forest pastures *etc*.) and 'Traditional hay meadows'. Pastures can be managed in 'Sole use' or have the status of 'Common land'.

The two following arguments justify defining a category 'Temporary grasslands':

- Grassland areas are often underestimated when they correspond to the category 'Permanent grassland and meadow' or even to the sum of the category 'Permanent grassland and meadow' and the category 'Fodder crops/Temporary grass'. 'Fodder crops/Leguminous plants' are usually not included in grasslands since they are supposed to not include grass!
- The current category 'Fodder crops/Leguminous plants' is unclear. According to the understanding of farmers and national statistical services, they can include pure forage legumes or legume-grass mixtures. There is indeed no clear difference between the following covers: 100% lucerne, 90% lucerne with 10% grass, 80% lucerne with 20% grass, *etc*. Moreover, a pure sowing of lucerne can include after some time a variable proportion of spontaneously grown grasses.

Regarding (red) clover-grass mixtures, the situation is even less clear since red clover is almost never sown in pure stand; it is almost always mixed with grass. Do they have to be classified in the category 'Fodder crops/Leguminous plants' or in 'Fodder crops/Temporary grass'? For farmers, pure lucerne, lucerne-grass mixtures and red clover-grass mixtures are all fodder crops. They use one or the other according to soil characteristics, their experience and other factors. If red clover-grass mixtures are classified in 'Fodder crops/Leguminous plants' the problem is that after 2-3 years, they can include much more grass than clover!

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[4] The term 'sole use' is used for land that is not common.

According to countries, legume-grass mixtures can thus be included in the category 'Fodder crops/Leguminous plants' or in the category 'Fodder crops/Temporary grass'.

All these problems are removed if the two categories 'Fodder crops/Leguminous plants' and 'Fodder crops/Temporary grass' are replaced by 'Fodder crops/ Temporary grasslands'.

In order to take into account the variable proportion of legumes in temporary grassland swards, a simple 3-level system is proposed: pure legume sowing; legume-grass mixtures; pure grass sowing. This system could be replaced in the future by a new one with more precise assessment of the proportion of legumes in the sward. That could be done in a later document for temporary and permanent grasslands.

Classification based on all land use types of the Utilized Agricultural Area

1. Arable land
   1.1. Fodder crops
       1.1.1. Temporary grasslands
           1.1.1.1. Pure legume sowing
           1.1.1.2. Grass-legume mixtures
           1.1.1.3. Pure grass sowing
       1.1.2. Green cereals
           1.1.2.1. Green oats, barley, spelt, triticale, rye and other C3 cereals
           1.1.2.2. Green oats, barley, wheat and other C3 cereals mixed with other crop types like annual legumes (e.g. pea, vetch)
           1.1.2.3. Green maize and sorghum
       1.1.3. Fodder roots (including fodder beet)
       1.1.4. Fodder brassicas
       1.1.5. Fodder *Compositeae*: sunflower
   1.2. Fallow lands
       1.2.1. Grazed fallow lands
       1.2.2. Non-grazed fallow lands
   1.3. Other crop types
2. Permanent grasslands
   2.1. Agriculturally improved permanent grasslands[5]
   2.2. Natural and semi-natural grasslands
       2.2.1. Pastures, including rangelands, rough grazing, forest pastures, *etc*.
           2.2.1.1. Sole use
           2.2.1.2. Common land
       2.2.2. Traditional hay meadows
   2.3. Permanent grasslands no longer used for production
3. Permanent crops
4. Other agricultural land such as kitchen gardens

Multilingual vocabulary of grassland terms

Fodder areas: Futterflächen (DE); Superficies forrajeras (ES); Surfaces fourragères (FR); Superfici foraggere (IT); Voederareaal (NL); Plochy krmovín (SK); Foderarealer (SE)

Fodder crops: Futterpflanzen (DE); Cultivos forrajeros (ES); Cultures fourragères (FR); Colture foraggere (IT); Voedergewassen (NL); Krmoviny (SK); Fodergrödor (SE)

Grasslands: Grünland (DE); Pastos (ES); Prairies (FR); Prati e pascoli (IT); Grasland (NL); Trávne porasty (SK); Gräsmarker (SE)

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[5] Almost always in sole use but occasionally common land

Meadows: Wiesen (DE); Pastos de siega (ES); Prairies de fauche (FR); Prati da sfalcio (IT); Maailand (NL); Lúky (SK); Slåtterängar (SE)

Pastures: Weiden (DE); Pastos de pastoreo (ES); Pâtures, prairies pâturées (FR); Pascoli (IT); Weilanden (NL); Pasienky (SK); Betesmarker (SE)

Permanent grasslands: Dauergrünland (DE); Pastos permanentes (ES); Prairies permanents, Surfaces toujours en herbe (FR); Prati e pascoli permanenti (IT); Blijvend grasland (NL); Trvalé trávne porasty (SK); Permanenta gräsmarker (SE)

Agriculturally improved permanent grasslands: Landwirtschaftlich entwickeltes Dauergrünland (DE); Pastos mejorados (ES); Prairies permanentes améliorées (FR); Prati e pascoli permanenti migliorati (IT); Landbouwkundig verbeterd blijvend grasland (NL); Intenzifikované trvalé trávne porasty (SK); Förbättrade permanenta gräsmarker (SE)

Natural and semi-natural grasslands: Natürliches und naturnahes Dauergrünland (DE); Pastos naturales y seminaturales (ES); Prairies naturelles et semi-naturelles (FR); Prati e pascoli naturali e semi-naturali (IT); Natuurlijk en half natuurlijk grasland (NL); Prírodné a poloprírodné trávne porasty (SK); Naturbetesmarker och hagmarksbeten (SE)

Permanent grasslands no longer used for production: Aus der Produktion genommenes Dauergrünland (DE); Pastos permanentes no utilizados para la producción (ES); Prairies permanentes plus utilisées pour la production (FR); Prati e pascoli permanenti non più utilizzati per la produzione (IT); Blijvend grasland dat uit productie is genomen (NL); Neprodukčné trvalé trávne porasty (SK); Permanenta gräsmarker tagna ur produktion (SE)

Rangelands: Rangelands, Hutungen (DE); Rangelands, Pastos de uso extensivo (ES); Parcours (FR); Rangelands, Spazi vasti a pascolo (IT); Rangelands, woeste gronden (NL); Rangelands, Extenzívne pasienky (SK); Rangelands, Extensiva betesmarker, Fjällbeten (SE)

Temporary grasslands: Wechselgrünland (DE); Pastos sembrados temporales (ES); Prairies temporaires (FR); Prati e pascoli temporanei (IT); Tijdelijk grasland (NL); Dočasné trávne porasty (SK); Vall på åker (SE)

Grazed fallow lands: Beweidete Brachen (DE); Barbechos y posíos (ES); Jachères pâturées (FR); Riposo pascolativo (IT); Braakliggende gronden die beweid worden (NL); Spásaný úhor (SK); Betad träda (SE)

Grazed common lands: Gemeinschaftsweiden (DE); Pastos comunales (ES); Terrains communaux pâturés (FR); Terreni a pascolamento collettivo (IT); Gemeenschappelijke weidegronden (NL); Obecné pasienky (SK); Betad allmänning (SE)

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Theme 5 submitted papers

Roles and utility of grasslands in Europe

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Abstract

This paper is a synthesis of the first deliverable of the MultiSward project 'Roles and utility of grasslands in Europe' (De Vliegher and Van Gils, 2010). This report inventories the importance and spatial localization of grasslands and their multiple functions that benefit humans. In addition to the production of herbage for livestock, grasslands contribute to erosion prevention, biodiversity maintenance of flora and fauna, carbon sequestration, clean surface water and groundwater, and they provide an attractive environment for recreation and leisure activities.

Keywords: animal production, biodiversity, grasslands , GHG, soil quality, spatial distribution

Introduction

Grasslands are the main survival resource for about one billion people worldwide. In industrialized Europe, permanent (33%) and temporary (6%) grasslands cover some 39% of the agricultural area and form the basis of a strong ruminant livestock sector. Next to this, grasslands perform a broad range of functions that benefit humans. In Europe, pressure on land use is high and it is important to establish the possibilities and constraints of combining the functions of grasslands. The first deliverable in the MultiSward project 'Roles and utility of grasslands in Europe' (De Vliegher and Van Gils, 2010) inventories the spatial localization of grasslands and determines the importance, roles and utility of grasslands in Europe.

Grassland area and distribution

The European grassland area has been significantly reduced during the last 30 years as a result of intensification of grassland and animal production, decrease in cattle population, use of concentrates and soybean in the ration, abandonment, and the effect of EU-policy (Huyghe *et al*., 2014). Nevertheless, grasslands still cover the largest proportion of the agricultural area. Permanent grassland is very important in Ireland (75% of UAA), UK (58% UAA), Slovenia (58% UAA) and Austria (55% UAA). In terms of number of hectares the United Kingdom (11 million ha), France (9.8 million ha), Germany (4.8 million ha), Italy (4.5 million ha) and Romania (4.5 million ha) represent 62% of the total permanent grassland area in EU-27. The percentage of UAA used as grassland varies considerably between countries and regions. Grassland productivity is affected by several factors: soil characteristics, climatic conditions - particularly total and seasonal distribution of rainfall and temperature - altitude, latitude and management.

Animal production

The principal aim of most European grasslands is to support animal production for milk and meat. The grazing livestock (78.210 million LSU) in EU-27 is divided as follows: 82% are cattle, 13% are sheep and goats and 5% are equidae. In EU-27, 75% of the cows are dairy cattle and 25% are meat cattle. Dairy and meat sector varies strongly from region to region. The top 20% of dairy products is produced on 5% of the territory and the last 10% is produced on 24% of the EU-territory. Sheep and goats are mainly concentrated in the Mediterranean countries, the United Kingdom and Romania. Equines are more common in central and northern Europe. Grazing livestock density is an indicator of the intensity of grassland use and of the pressure of livestock farming on the environment. Manure produced by livestock contributes to greenhouse

gas emissions and nutrient leaching into water and air. Higher density means a higher amount of manure per ha UAA, which increases the risk of N-leaching. An excessively low livestock density increases the risk of land abandonment and increases the need for industrial fertilizers. Next to grazing livestock, farming practices also influence environmental impact.

Organic farming has increased significantly in the period 2000-2011 to 4.1% UAA in EU-27. It is particularly present in regions with extensive livestock production systems based on permanent grasslands. This concerns mountainous and semi-mountainous regions in alpine areas and other parts of the EU. Strikingly, the significance of permanent grassland represents 33% of the UAA in EU-27, whereas it represents 47% of the whole organic area (Anonymous, 2013).

Energy production and biorefinery

The increasing cost of fossil fuels and environmental concerns about climate change also influence crop-based agro-fuel production and demand. Grassland and fodder area competes with arable land for first-generation bio-fuels like ethanol (maize, wheat, barley, sugar beet), bio-diesel (oilseed rape extraction) and methane (biogas maize). Combustion of grassland biomass is less favourable than other crops or residues like straw because of the NO_x, SO_2 and HCl emissions.

Biorefinery is a concept using green biomass (pasture) as raw material to produce high value biochemicals from the liquid fraction and lower value products or energy generation from the grass-fibre fraction. The grass resource could be natural or cultivated grassland or verge grass that is not needed for traditional use (i.e. forage for herbivores). The general challenges in biomass processing are the transportation costs, the use of dry or wet products, the choice of a central or a mobile unit, and the choice between storage for a year-long period versus a campaign during the growing season.

Soil quality and protection

Grasslands act as a carbon sink. Several studies have shown a steady increase of soil organic carbon in grassland soils, where over time the carbon levels rise above those of arable soils. However, carbon losses also occur much faster after ploughing up the sward. This highlights the importance of conservation of grassland surfaces and sward longevity for climate-change mitigation. On the other hand, emissions of N_2O from grassland soils and manure deposition and CH_4 from grazing ruminants partially counterbalance the mitigating effects of carbon sequestration.

Grasslands can also mitigate soil erosion and pollution. Grasslands provide a dense rooting system and a permanent soil cover. Ploughing grasslands is seen as one of the causes of increased erosion problems in some European regions. In general, pesticide use and risk of environmental pollution are much lower in grassland systems compared to annual (forage) crops. Nutrients and pollutants left on the grassland surface decompose quickly due to an intensive biological activity. Grasslands thus act as a biological filter for the migration of various chemicals towards the surface and groundwater systems.

Grasslands and biodiversity

One of the most important functions of (semi-natural) grasslands in Europe is supporting high biodiversity. Grasslands are crucial not only for a great variety of plant species but also for many species of farmland birds, butterflies, beetles, *etc*. Many species of grasslands are rarely found in other vegetation types. Through variations in management style, climatic and abiotic conditions, semi-natural grasslands show a great variety. Grassland plant communities in the EU are classified into seven main habitats according to EUNIS (2006): dry grasslands, mesic grasslands, seasonally wet and wet grasslands, alpine and subalpine grasslands, woodland fringes and clearings and tall forb stands, inland salt steppes, and sparsely wooded grasslands.

Intensification of land use poses a threat to the botanical diversity in grassland swards, but so does land abandonment, related to the phenomenon of rural abandonment or abandonment of parcels that are of little agricultural value (e.g. on steep slopes or in marginal areas). Ceasing grassland management means that vegetative succession then progresses, with the encroachment of shrubs and other woody species leading to the disappearance of many typical grassland species. Soil biota play an important role in (grassland) ecosystem services and production, e.g. water regulation, nutrient supply. In arable fields the accent is more on bacterial communities whereas fungi fulfil a more important part in grassland soil ecosystems with increasing populations and genetic diversity with sward age. Last, grasslands contribute to an attractive landscape as they are perceived as a rather natural landscape feature and often preferred over other land use such as settlements or arable fields. Especially semi-natural grasslands tend to improve the 'naturalness' of a landscape as they show an increased colour and structure. For this reason, grassland areas are beneficial for tourism and outdoor recreation.

Conclusion

Grasslands always combine several functions but in different ratios depending on local situations. In addition to the production of herbage for livestock, grasslands contribute to the maintenance of biodiversity, sequester carbon into soil, clean surface and groundwater, prevent erosion and provide an attractive environment for recreation and leisure activities.

Acknowledgements

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An indicator-based tool to assess environmental impacts of multi-specific swards

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Abstract

We developed a set of indicators to assess the impacts of grassland plant diversity and management on the abundance and diversity of six animal taxa (biodiversity indicators), on soil, water and air quality, and energy consumption (abiotic indicators). The methodology combines multi-criteria decision trees with fuzzy partitioning, allowing to deal with different types of information (qualitative or quantitative, more or less accurate knowledge). Biodiversity indicators were calculated from simple and easily accessible input variables (main management features and botanical composition), according to an analysis of the literature, and could be partly validated at plot scale. Abiotic indicators were calculated as model outputs at farm scale. Combining these two types of indicators allows assessment of the overall environmental footprint of grassland management practices and discussion of the benefits provided by multi-species swards. Here, we report major advances and obstacles closely linked to the available scientific and technical knowledge.

Keywords: grassland, diversity, management, indicator

Introduction

For several decades grasslands have been gradually decreasing across Europe, and many remaining grasslands have become simplified to grass monocultures or simple grass-clover mixtures. On the other hand, research in ecology has shown the benefits of plant diversity for the functioning of grassland ecosystems. This stresses the need for increasing knowledge of the positive economic and environmental values of multi-species swards in agricultural systems. In agriculturally used grasslands, plant species richness varies from simple grass-clover mixtures to semi-natural grasslands that may contain up to 50 species per plot. A key objective of the European research project FP7 'MultiSward' was to conceive, evaluate and promote sustainable ruminant production systems based on multi-species grasslands. One step to reach this objective was the elaboration and the validation of an operational evaluation tool (OET) based on a set of indicators sensitive to plant diversity and grassland management.

Materials and methods

The development of the OET was based on three sources: 1) expert consultation to decide key environmental impacts and the structure of the OET, 2) analysis of scientific literature to determine how plant diversity impacts on environmental outputs, and 3) real datasets used to either calibrate or validate the OET. Fifteen experts from five countries (CH, F, IRL, N, NL) were involved in the procedure. The OET consists in a decision tree where leaves reflect individual environmental impacts (e.g. nitrate in soil water or web-spider abundance) and branches or nodes reflect aggregated impacts (e.g. soil water quality or spider diversity). The calculation of basic (at leaf level) or aggregated (at node level) indicators results in a score between 0 and 10, (10 corresponds to an extremely favourable environmental impact as compared with other types of grassland management). Elaboration of the tree was performed with a qualitative multi-criteria decision modelling and support system called FisPro (Suárez

and Lutsko, 1999). It implements a decision tree with fuzzy partitioning model, which makes it possible to account for uncertainty in the decision boundaries between alternatives. Therefore, the nodes can present different kind of data-sources and data uncertainty.

Results and discussion

The complete evaluation tool is presented in Figure 1.
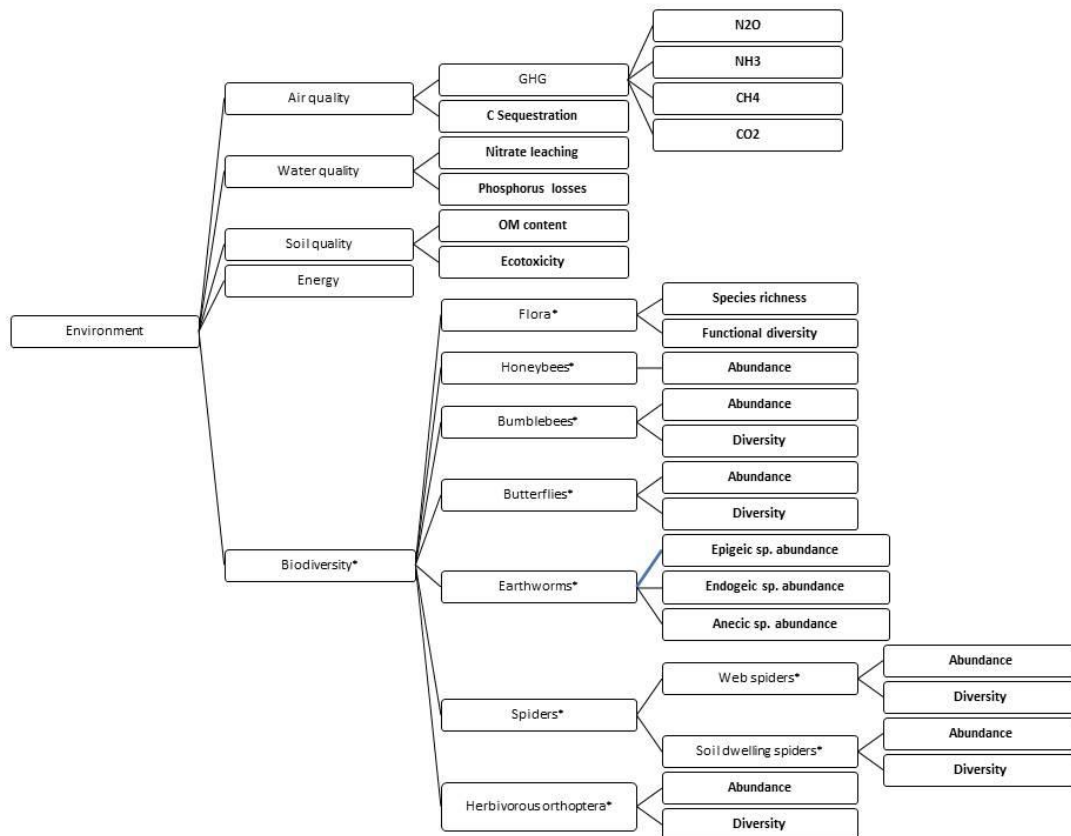
Figure 1. Structure of the indicator-based tool for environmental assessment in MultiSward. Basic indicators in bold, aggregated indicators followed by a star.

We initially aimed at calculating all indicators at plot and farm level, but literature analysis and expert consultation showed two major obstacles: 1) the lack of knowledge on the effects of sward plant diversity on 'abiotic impacts' (air, soil, water quality and energy) and 2) the lack of dataset to calibrate and validate biodiversity indicators at farm scale. Nevertheless, the OET in its current form calculates all biodiversity indicators at plot scale, taking account of the effects of sward diversity and management. Abiotic indicators can be calculated at farm scale, taking into account management factors but, very poorly, sward diversity. Indicator inputs are made of discrete or continuous management variables, soil features and botanical composition. Outputs (basic indicators) are then calculated either by models DairyWise (Schils *et al*., 2007) and Melodie (Chardon *et al*., 2012) for abiotic indicators, or by the newly developed decision tree for biodiversity indicators. As an example, Table 1 shows the impact of sward diversity and management on two biodiversity indicators calculated for 395 French grasslands across a wide range of pedoclimatic conditions, and housing between 1 and 69 plant species.

Table 1. Biodiversity indicator (0 low – 10 high richness and abundance) for two taxa calculated on a set of 395 French grasslands. Sp = species richness per plot . TG or PG= Temporary or Permanent Grassland

Management	Plant diversity	Spiders diversity and abundance	anecic earthworms abundance
continuous grazing with medium stocking rate	TG	3.40	6.56
	PG <20 sp	5.99	6.01
	PG 20-35 sp	6.30	3.99
	PG 36-50 sp	6.60	4.13
	PG >50 sp	6.15	3.83
Rotational grazing with low stocking rate and low or high grass height after grazing period	TG	5.88	5.35
	PG <20 sp	5.76	3.41
	PG 20-35 sp	6.15	4.29
	PG 36-50 sp	6.32	3.96
	PG >50 sp	6.82	4.85
One cut and rotational grazing, short duration of grazing periods	TG	2.76	5.35
	PG <20 sp	2.77	4.85
	PG 20-35 sp	2.88	4.62
	PG 36-50 sp	2.91	5.53
	PG >50 sp	N/A	N/A

A first run of validation with real data (indicator value *vs* biodiversity observations) and with expert opinions on outputs showed promising results. Nevertheless, the main restriction encountered during the development of the OET was the lack of knowledge, which in turns encourages further basic research on grassland functioning.

Conclusion

The OET developed in the MultiSward project is the first set of 'pressure indicators' (*sensu* DPSIR indicators typology of the European Environment Agency) sensitive to management and sward plant diversity. Indicators do not have to be compared to models outputs, as the main goal is not to predict a precise and real value but get a score which permits the right decision for the decision maker. The calculation of indicators is based on simple and easily accessible inputs, and its implementation in a free website (http://eflorasys.inpl-nancy.fr) is in progress. The current state of knowledge makes it difficult to fully calibrate and validate all abiotic and biodiversity indicators at plot and farm levels. First results obtained on fauna biodiversity at plot level reveal the usefulness of including plant diversity and simple management inputs to improve the environmental evaluation of grassland based systems.

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Assessment of ecosystem services provided by grasslands and grassland-based systems by indicators: a regional perspective

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Abstract

The many ecosystem services that grasslands and grassland-based systems can provide should be better quantified in indicator systems. The MultiSward Indicator System (MIS) is inspired by the agri-environmental indicators of the European Commission (EC) calculated at country level. Its structure is based on the DPSIR framework. The MIS focuses on grassland-based ruminant systems. Its scope is thus more restricted than the agri-environmental indicator system of the EC but it tries to be compatible with this system. The work is based on data that are available within EC institutions. The MIS includes two lists: the first one is calculated per farm type for a selection of regions and includes 21 indicators, and the second one is calculated per region for the same selection (all farm types merged) and includes 45 indicators. The MIS should be considered as a pilot project. It should be extended in the future to all European Union regions.

Keywords: indicator, ecosystem service, region, livestock, grassland-based systems

Introduction

Indicators are necessary for justifying, designing and assessing agricultural and environmental policies. There is an obvious lack of indicators for quantifying ecosystem goods and services in grassland-based systems. That is why an indicator set was developed at regional level in the FP7 project 'MultiSward'. Its objective is: (i) to evaluate the impacts of ruminant stockbreeding systems on the quality and use of natural resources (air, water, soil, energy, biodiversity), and (ii) to assess a large range of ecosystem goods and services provided by grasslands and grassland-based systems. The Multisward Indicator System (MIS) aims to assess these impacts and the provision of these ecosystem goods and services for comparing: (i) ruminant production systems between them and with other farming systems like specialist field crops, within regions, (ii) regions with different proportions of permanent and temporary grasslands, green maize and arable crops in their agricultural area.

Materials and methods

At region level, the MIS is inspired by recent and effective systems, and particularly by the 28 agri-environmental indicators of the EC (European Commission, 2006) calculated at country level. Its structure is based on the DPSIR (Driving forces – Pressures – States – Impacts – Responses) framework of the European Environment Agency (EEA, 1999). The MIS focuses on grassland-based ruminant systems. Its scope is thus more restricted than the agri-environmental indicator system of the European Union but it tries to be compatible with this

system. The work is based on data that are in their majority available within EC institutions to ensure that other actors could use this indicator system in the future.

Table 1. List of indicators evaluated at region level.

Responses	**Public policy**
	1. Share of agri-environment payments in gross farm income (%)
	2. Agricultural areas under Natura 2000 (%AA)
	Technology and skills
	3. Farmers' training levels and use of environmental farm advisory services
	Market signal and attitudes
	4. Area under organic farming
Driving forces	**Input use**
	5. Energy use
	Land use changes
	6. Evolution of the % of permanent grassland in the AA between 2000 and 2010 (%)
	Grassland and forage crop patterns
	7. Fodder crops and grass (%AA)
	8. Common land area (% PG)
	9. Total permanent grassland and meadow (%AA) (=PG)
	10. Temporary grass (%AA)
	11. Temporary grass + leguminous plants (%AA) (=TG)
	12. Total permanent and meadow + temporary grassland (%AA) (=MFA)
	13. Total permanent and meadow + temporary grassland + leguminous plants (%AA)
	14. Green maize (%AA)
	Livestock patterns
	15. Stocking rate (grazing livestock) (LU/ha of fodder crop and grass)
	16. Stocking rate (grazing livestock) (LU/ha of TG + PG)
	17. Stocking rate (total livestock) (LU/ha of total AA)
	18. Stocking rate (total livestock) (LU/ha of fodder crop and grass)
	19. Stocking rate (total livestock) (LU/ha of PG+TG) (2010)
	Farm management
	20. Grassland management (in %MFA)
	21. Share of grassland grazed (%)
	Trends
	22. Intensification / extensification
	23. Grassland annual yield assessment (t DM/ha) per grassland type
	24. Annual milk yield/cow (l/cow)
	25. Specialisation
	26. Risk of land abandonment
Pressures and	**Pollution**
	27. Gross nitrogen balance (kg N/ha) (total surplus)

benefits	28. Risk of pollution by phosphorus (kg P_2O_5/ha) (total surplus)
	29. Pesticide use (pesticide use in grassland/pesticide use in arable land) (%)
	30. Ammonia emissions (kg $N-NH_3$/ha)
	31. Greenhouse gas emissions (GWP, total CO_2 eq.) (kg CO_2 eq./ha)
	32. CH_4 emissions (kg CO_2 eq./ha)
	33. N_2O emissions (kg CO_2 eq./ha)
	Resource depletion
	34. Annual soil erosion risk by water (t soil/ha)
	Benefits
	35. High Nature Value grasslands (%AA)
	36. Number of N2000 grassland habitats
	37. Proportion of N2000 grassland habitats (%AA)
State / Impact	**Planned / Agricultural biodiversity**
	38. Shannon diversity index of land use type
	39. Shannon equitability index of land use type
	40. Shannon diversity index of grazing livestock species expressed in LU
	41. Shannon equitability index of grazing livestock species expressed in LU
	Functional biodiversity
	42. Biological nitrogen fixation in grasslands (kg N/ha)
	Heritage biodiversity
	43. Population trend of farmland birds
	Natural resources
	44. SOC density in grassland soils (t CO_2 eq./ha)
	Landscape
	45. Landscape - state and diversity (tree lines, hedges, stone wall) (%holdings)

Results and discussion

The MIS includes two lists: the first one is calculated per farm type for a selection of regions, and the second one is calculated per region for the same selection (all farm types merged). The lists evaluated per farm type and per region include, respectively, 21 and 45 indicators. The MIS is quantified in four (Atlantic, Continental, Alpine, Mediterranean) of the five main European biogeographical regions and in 12 administrative regions selected according to a typology of livestock regions based on Pflimlin *et al.* (2005). The list of indicators evaluated at region level is presented in Table 1.

Conclusion

The MIS is developed and ready for use. It includes 45 indicators calculated at region level and 21 indicators calculated at farm type level within regions. It is able to compare individual NUTS2 regions, averages per NUTS2 regions and farm types. Indicators have proved to be sensitive. They correspond to a wide range of topics. The MultiSward and the EC indicator lists are not identical. They have their own specificities and are complementary. The MIS should be considered as a pilot project, but the indicator sets should be calculated in the future in all European Union regions. In the future, efforts should continue on data collection (e.g. on field

and by remote sensing), prediction (models) and development of missing indicators (water percolation; water use; water quality [nitrate, pesticide]; vegetation types of grasslands; proportion of legumes in temporary and permanent grasslands; grassland yields per vegetation type; grassland management).

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Threats and opportunities for European grassland areas under different market and policy scenarios

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Abstract

Grasslands are an important landscape element in Europe, but have seen a reduction in area in the last 50 years. To assess future changes under varying price and policy scenarios the FARMIS agri-sector model was utilized to model likely farmer behaviour with regard to grassland use. The results indicate that the total area of grasslands and specifically extensive grassland can be improved if relevant output prices increase or support payments are transferred from arable to lowland/ hill pastoral farms.

Keywords: grassland, scenarios, FARMIS, FADN, Switzerland, Germany, Wales

Introduction

Grasslands are an important landscape component in Germany (DE), and characterize the cultural landscapes of Wales (CY) and Switzerland (CH), covering 30%, 85% and 70% of agricultural land respectively. From an ecological point of view, grassland-based production systems have numerous advantages compared to pure arable or forage crop systems. They provide environmental benefits through capacity to reduce flooding, act as a carbon sink, support biodiversity and offer landscape amenity which can embody a range of values important to society (Bellarby *et al.*, 2008; Dillon, 2011). Furthermore, areas under grasslands can be used exclusively for feeding ruminants, avoiding competition with humans for grains and pulses (Peeters, 2011). The question is how to maintain or increase grassland areas of Europe.

Materials and methods

The objective of this paper was to assess a range of price variations and policy scenarios utilizing the FARMIS agri-sector model to identify trends and effects. FARMIS is a comparative-static process-analytical programming model for farm groups (Offermann *et al.*, 2005; Deppermann *et al.*, 2013), in which production is differentiated for 27 crop and 15 livestock activities. The model specification is based upon information from the farm accountancy data networks (FADN), supplemented by data from farm management manuals where FADN lacks detail such as feed or fertilizer quantities. Key characteristics of FARMIS are: 1) The use of aggregation factors that allow for representation of the sectors' income; 2) input-output coefficients which are consistent with the farm accounts; and 3) the use of a positive mathematical programming procedure to calibrate the model to the observed base year levels. Within this paper, four scenarios were analysed to assess the effects of selected changes in market or policy conditions on a grassland based farming systems. The two market scenarios assessed the effects of a 50% increase in the price of inputs (IP_+50) or outputs (OP_+50) relevant to grassland farming. The considered policy scenarios had two common themes, a) '+ grass payment' considered a greater payment to permanent grassland, in DE with re-allocation of budgets, in CY and CH with a 'top up' payment, b) '+ ext. grass, - arable payment' considered an extra payment to extensive grassland, with reduced payments to arable land.

Results and discussion

Impacts of these four scenarios on production, land use and income can be seen in Table 1. In general, increased input costs (output prices) caused a reduction (increase) in arable and intensive grassland areas but an increase (decrease) in extensively managed grassland areas. In some regions of Germany these decreases caused high rates of grassland abandonment (about -50% Schleswig Holstein and Lower Saxony) due to high output prices. In Switzerland a reduction of intensively managed grassland areas in favour of temporary grasslands was observed, but generally Swiss farmers reacted less intensively to price changes. Additional payments to grassland achieved small effects in Germany and Wales, but a much greater effect in Switzerland, linked to the higher ratio of support payments to production value of farm output. When a greater emphasis was placed upon payments for extensive grassland, at the expense of payments to arable land, a stronger impact was seen in Germany and Switzerland. In Wales, the area of arable land for reallocation of budget was minimal, causing lower payments to intensive grassland.

Table 1. Impact of different market and policy scenarios on land use, production and income at the sector level in Switzerland (CH), Germany (DE) and Wales (CY)

	IP_+50			OP_+50			+ grass payment			+ ext. grass, - arable payment		
	CH	DE	CY	CH	DE	CY	CH	DE	CY	CH	DE	CY
						% Change to Baseline						
Arable land	-2	-2	-3	3	0	0	-18	0	0	-40	0	-4
Level of arable grass, leys	-3	-4	-5	6	41	16	-48	-1	0	-91	9	-9
Level of fodder maize	-4	-5	-8	30	22	18	0	0	0	10	0	0
Permanent Grassland	0	-2	-7	0	0	2	0	0	2	7	0	-1
Level of intensive grassland	0	-10	-14	-2	16	25	21	0	1	46	-30	0
Level of extensive grassland	4	18	5	0	-42	-55	2	1	8	23	80	-5
Number of dairy cows	-4	1	-9	51	29	29	0	0	0	14	-1	0
Number of suckler cows	1	-4	-1	21	-2	20	0	0	2	11	-11	-2
Number of beef cattle (CH), bulls (DE), ewes (CY)	-12	-19	-22	37	22	12	0	0	2	-4	-1	-2
Production of beef (CH, DE), lamb (CY)	-7	-12	-6	43	17	13	0	0	1	6	-2	-1
Production of milk	-4	1	-9	51	29	29	0	0	0	15	-1	0
Production value total	-2	-6	-8	63	37	93	0	0	1	7	-1	-1
Total Subsidies	0	-2	-7	3	1	2	7	-1	91	-4	1	0
Net added value at factor prices per AWU	-8	-28	-97	81	69	378	8	0	156	4	3	3
Family Farm income + wages per AWU	-10	-24	-162	105	81	629	10	0	220	6	0	11

Source: Own illustration based on FARMIS 2013

The economic effect of price-change scenarios illustrates how much input prices reduce output (through reduced intensity of farming) and how profitability is influenced negatively. For instance, the number of bulls in Germany, the number of ewes in Wales and the number of beef cattle in Switzerland decreased by -19% and -22% and -12% respectively. The increased output prices have the opposite effect and induce large increases in production value and profitability, though this increase in production has negative environmental effects through greenhouse gas emissions and nutrient balances. The economic effects of policy scenarios highlight how additional grassland payments led to small changes in economic values in Germany and partly

Switzerland. In Wales the scenario '+grass payment' exceeds CAP budgets (+91%), had a massive effect on profitability (+156%), but only achieved minimal gains in extensive grassland areas (+8%). A support of extensive grassland (+ext. grass, - arable payment) achieved slight gains in profitability at sector level through transfer of income from the stronger arable sector to less profitable extensive grassland sector in all three countries. These policy scenarios had a minimal environmental effect in Germany and Wales, but in Switzerland increased numbers of ruminants caused increased greenhouse gas emissions but reduced nitrogen eutrophication.

Conclusion

In conclusion, it can be seen that input and output prices have a significant effect on the area and intensity of grassland systems. Use of support payments to encourage less-intensive grassland farming appears to achieve its goal when these payments are transferred from arable areas, but may be more difficult to achieve when there is a limited arable area to transfer budget from, as demonstrated by results in Wales. To prevent extensive grassland abandonment, an increasing proportion of support-payment budgets may need to be transferred from arable and lowland farms to upland areas. In general, policy effects at farm-type level can often vary due to varying intensities of grassland farming.

Acknowledgement

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Appreciation of the functions of grasslands by European stakeholders

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Abstract

The European project MultiSward aimed to increase the reliance of farmers on grasslands and on multi-species swards for competitive and sustainable ruminant production systems. Active participation of stakeholders was one of the key objectives of the project. The aim of the current study was to get an insight into the importance of grasslands for stakeholders in Europe. An on-line questionnaire on the functions of grasslands was developed in eight languages and 1959 valid responses were obtained. Belgium, France, Ireland, Italy, the Netherlands and Poland were the countries with the highest responses. All of the stakeholder groups that were identified as being important in the stakeholder analysis responded to the questionnaire. When asked about the importance of different aspects of sustainability, stakeholders, on average, valued economic aspects the highest, followed by ecological aspects and finally, social aspects. There were, however, differences between countries and stakeholder types. The results of the questionnaire show that individual functions of grasslands are highly recognized and appreciated by all relevant stakeholder groups. We conclude that the large European grassland area is considered by all stakeholders to be a valuable resource that is essential for economy, environment and people.

Keywords: grasslands, multifunctionality, stakeholder, sustainability, questionnaire

Introduction

Grasslands, with their multifunctional roles, can provide a good basis for developing sustainable production systems in the long term (Peyraud *et al.*, 2010). The project MultiSward (www.multisward.eu, 2010-2014) aimed to secure optimal acreage and utilization of grasslands in Europe, to highlight the benefits of grasslands and to conceive, evaluate and promote sustainable ruminant production systems, based on the use of grasslands with a high level of multi-functionality, to increase simultaneously the competitiveness of ruminant production systems and provide environmental goods and biodiversity preservation.

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 can be of crucial importance for preserving biodiversity, protecting soils against erosion and maintaining the local population density. The reduction has differed between countries. Losses were high in Belgium, France, Italy and the Netherlands while the grassland area remained almost stable in Luxembourg and the United Kingdom. In 2007, permanent grasslands covered over 57 million ha in the EU-27 and temporary grasslands about

10 million ha, which represents 33% and 6%, respectively, of the total utilized agricultural area (UAA) in the EU-27.

In order to contribute to the overall objective of MultiSward, stakeholder requirements and expectations with respect to multi-functionality of grasslands within Europe should be known, because a better understanding of stakeholders' perspective of grasslands leads to a better understanding of the importance of grasslands. Prior to the MultiSward project, the requirements and expectations of stakeholders with respect to the multi-functionality of grasslands in Europe were not known. Therefore, an active participation of stakeholders was one of the key objectives of the MultiSward project. An initial inventory was made of the requirements and expectations of stakeholders with respect to the multi-functionality of grasslands in Europe (Van den Pol-van Dasselaar *et al.*, 2012 and 2013). The aim of the current study was to give new insights into the importance of grasslands for stakeholders in Europe.

Materials and methods

An international team of representatives from Ireland, the Netherlands, France, Italy and Poland was established representing Atlantic, Mountainous, Mediterranean and Continental regions. The work started with a stakeholder analysis (Pinxterhuis, 2011). The identification of stakeholders is an important first step in stakeholder consultation. Stakeholders are usually defined as those who either affect or are affected (e.g. Freeman, 1984). In the case of grasslands, this means that stakeholders are those who affect grasslands or are affected by grasslands. Both aspects were taken into account when prioritizing the stakeholders in the stakeholder analysis. A good stakeholder analysis is essential (Reeda *et al.*, 2009), since only by understanding who has a stake in grasslands, can the appropriate stakeholders be effectively involved in the stakeholder consultation. The 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 new management options for multi-species swards (e.g. can directly benefit, has political power, is executing governance, is economically dependent, *etc.*). The most important stakeholders were the traditional foursome of primary producer, policy maker, researcher and advisor. NGOs for nature conservation and for protection of the environment were also considered important, together with industry (mainly processing and seed industry) and education. Following the initial stakeholder analysis, the international stakeholder team undertook several studies, including national and international meetings.

A questionnaire on the functions of grasslands was developed in eight languages: Polish, Dutch, Italian, French, English, German, Danish and Swedish, using SurveyMonkey (www.surveymonkey.com). The questionnaire included two main questions on the importance of grasslands in Europe. First, respondents were asked for their opinion on sustainability. This term covers economic, environmental and social issues (profit, planet, people). Respondents to the questionnaire were asked to divide 10 points across these three aspects of sustainability, giving most points to the one they considered the most important aspect (e.g., 4, 3, 3 if they considered that ecological and social aspects are of equal interest and that economy is slightly more important). Second, the respondents were asked to score 42 predefined functions of grasslands of grasslands for importance in their region (1 = not important; 5 = very important). These functions are examples of the ecosystem services that grasslands deliver. The concept of ecosystem services provides a good insight into the benefits that humankind gains from its interaction with natural resources, in this case with grasslands. The Millennium Ecosystem Assessment report (MEA, 2005) distinguishes four groups of ecosystem services: (i) provisioning services: products obtained from ecosystems, e.g. production of food, water, (ii) regulating services: benefits obtained from the regulation of ecosystem processes, e.g. control of climate and disease, (iii) cultural services: non-material benefits that people obtain from

ecosystems through spiritual enrichment, cognitive development, reflection, recreation, and aesthetic experiences, e.g. recreation and beauty of the landscape, and (iv) supporting services: ecosystem services that are necessary for the production of all other ecosystem services, e.g. nutrient cycles, crop pollination.

Research partners of MultiSward actively distributed the questionnaire in Europe to stakeholders. Furthermore, several relevant associations with members from different stakeholder groups were approached, such as the national Grassland Societies in the respective countries. The questionnaire was available online from spring 2013 and closed at the end of 2013.

The sustainability results were analysed using GenStat (VSN International, 2013). The observed points out of a total score of 10 have been treated as pseudo-binomial data, taking the variance to be proportional to binomial variance (McCullagh and Nelder, 1989). Differences between countries, stakeholder type, gender and age in preference of the respondents have been assessed by linear logistic regression analysis of the observed points using a logistic model with main effects. Main effects have been tested with approximate F-tests; differences between countries, stakeholder type, gender and age have been tested with approximate t-tests on all pairwise differences of fitted marginal means on the underlying logistic scale.

Results and discussion

At the time of closing the questionnaire, 1959 valid responses had been obtained for the question on sustainability aspects. The majority of respondents (1798) also provided answers to the question on the different functions of grasslands. The respondents originated from 27 different countries in Europe. There were six countries with more than 200 responses: France (21% of the total responses), Italy (17%), Ireland (13%), Poland (12%), Belgium (11%) and the Netherlands (11%). The remaining countries in the rest of Europe were grouped (15%). All the relevant stakeholder types described in Pinxterhuis (2011) responded to the questionnaire. Responses from researchers, advisers and farmers accounted for a high proportion of the total: 22%, 19% and 17% of the total responses, respectively. The contribution of policymakers was much lower (6%), but given the fact that there are obviously fewer policy makers and they are often less eager to respond, we were satisfied with this percentage. Other groups were students (16%), educators (6%), industry (5%), e.g. feed industry, dairy industry, seed industry, and finally NGOs (3%). The remaining group, which mainly consisted of people who identified themselves as consumers, press, in between jobs *etc*. was 6%. Some people identified themselves as belonging to two groups. In those cases, they were classified into the group which they mentioned first. With respect to age and gender, responses were obtained from all age categories. One-third of the respondents were female and two-thirds were male. The percentage of female respondents in the younger age groups was higher than the percentage of female respondents in the higher age groups. Finally, it was observed that the majority of the respondents had received a high level of education, as two-thirds of the respondents had attended university. It is to be expected that respondents in a number of stakeholder groups have a position that requires a relatively high level of education. The groups 'farmers', 'students' and the 'rest' group had a lower level of education. A further explanation might be that well educated people may be more willing to respond to a questionnaire.
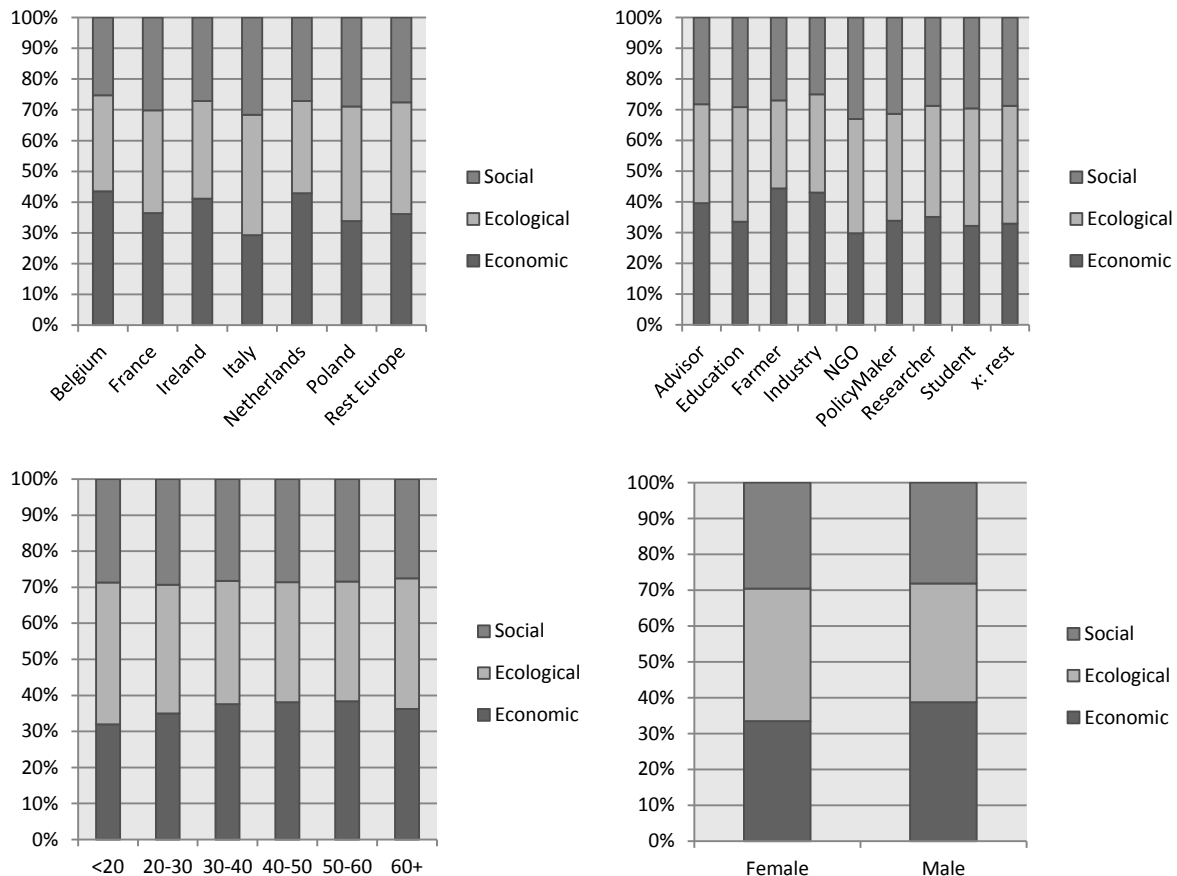
Figure 1. Importance of economic, ecological and social aspects of sustainability for a) different countries, b) different stakeholder types, c) different age and d) different gender (total of economic, ecological and social aspects equals 10 for each group) (n=1959).

When people were asked to divide 10 points over economic, ecological and social aspects of sustainability, on average, economy was valued the highest (3.7) followed by ecology (3.4) and social aspects (2.9). The differences were significant, but these means also show that all aspects of sustainability were considered to be important. The effect of country, stakeholder, age and gender is shown in Figure 1. Obviously, respondents only had 10 points to divide. This means that the effects on economic, ecological and social aspects are entangled. When a respondent, for instance, decides to give more points to social aspects, there will be fewer points left for the other two aspects. We therefore looked for pairwise significance. When analysing economic, ecological and social aspects, the effects of country and stakeholder type were significant ($P<0.001$). The effect of age and gender was less consistent; after having accounted for the remaining main effects of country and stakeholder type, the age and gender effect was often no longer significant.

Italy showed the lowest ranking for economy, followed by Poland and France (Figure 1a). Belgium, Ireland and the Netherlands had a high ranking for economy. In accordance with this, Italy, France and to a lesser extent Poland, showed higher ranking for social aspects than the other countries. Ecological aspects were scored highest for Italy and Poland. Concerning the different stakeholder types (Figure 1b), farmers, industry and to a lesser extent advisers, showed the highest ranking for economy; the social aspects were valued the highest by NGOs and policy makers and lowest by industry. Ecological aspects were valued highest by education, research and students, and lowest by farmers. There was hardly any difference in the ranking of social aspects in relation to age (Figure 1c). It seems that economy is ranked a bit higher when people get older at the cost of ecological aspects. However, differences were not significant. Females ranked economy lower than males mainly to the benefit of ecological aspects (Figure 1d).

When people were asked to value different functions of grasslands, it was clearly shown that the different functions of grasslands are highly recognized and appreciated by all relevant stakeholder groups (see papers on appreciation of the functions of grassland by Belgian, Dutch, French, Irish, Italian and Polish stakeholders elsewhere in this volume, and Van den Pol-van Dasselaar *et al*. (2014) for a summary of all results). It is therefore important that future policies continue to support the conservation of grasslands. Scenarios with less grassland will lead to an overall decrease in total ecosystem services delivered, since grassland is the only land-use option which is capable of delivering that large a number of ecosystem services simultaneously.

Conclusion

MultiSward provided an insight into the appreciation of the different functions of grasslands in Europe. It clearly showed that the different functions of grasslands are highly recognized and appreciated by all relevant stakeholder groups. The large European grassland area appears to be essential for economy, environment and people. We conclude that all stakeholders consider grasslands to be a valuable resource in Europe. Maintaining or increasing the grassland area and thus securing the importance of the different functions and services of grasslands in Europe is a challenge for the coming years. It is, however, important since it will ensure the continuation of different ecosystem services being delivered simultaneously by multifunctional grasslands.

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Theme 5 posters

Effect of grassland management in autumn on the mineral N content in soil

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Abstract

In this experiment we studied whether cutting pastures in autumn affects the mineral N content in the soil profile. These results were compared with soil-N content after grazing. The intention was to evaluate whether this may reduce the risk of N leaching. Twenty-seven parcels with an intensive, mixed management were selected in 2010-2012. Two cutting frequencies were applied: a single cut in October (n=27) or a cut in September and in October (n=15). Cutting in October instead of grazing resulted in a significant decrease in NH_4-N content in the 0-30 cm soil layer in October and November and a significant decrease in total mineral N content in the 0-30 cm soil layer in November. NO_3-N, which is sensitive to leaching, was not significantly influenced in this period. Cutting twice versus once had no significant effects on NO_3-N, NH_4-N, or mineral N content in the soil.

Keywords: grazing, cutting, intensive management, mineral N content soil

Introduction

Dairy farming in Flanders, Belgium is intensive and farmers are allowed to apply 235 kg $N_{available\ e}$.ha^{-1} $year^{-1}$ on grassland on sandy soils (245 kg on non-sandy soils) under the terms of the Flemish Manure Decree. Included within this definition of fertilization are slurry, chemical fertilizers and dung and urine deposited during grazing. The level of nitrate-N residue in the soil (0-90 cm) at the end of the growing season is used as an indicator for the risk of nitrate-N leaching. The aim of the experiment was to study the effect of cutting in autumn in comparison with grazing to evaluate the effect on mineral N content (nitrate-N and ammonium-N) in the soil profile. Our intention was to evaluate whether the risk of N leaching could be reduced.

Materials and methods

The study was performed in Flanders on permanent grasslands dominated by perennial ryegrass (*Lolium perenne* L.). In this experiment, 27 parcels with mixed management from the beginning of the season until the end of August were selected on intensive dairy farms. Some pastures were followed during 2 or 3 years. Three pastures per soil texture – sand, sandy loam and clay- were examined per year. No slurry or chemical fertilizers were applied later than 1 September. The pastures were mainly grazed by dairy cows in production - with supplementary feeding - or by heifers, until October-November. Grazing time, animal type and numbers of animals grazing were recorded, and N input from deposition of dung and urine was calculated (VLM, see below). In 2010 we set up 3 enclosures per pasture (4 m x 8 m) to prevent grazing on that area for the rest of the growing season. In 2011 (9 parcels) and 2012 (6 parcels) the enclosures had a larger area (6 m x 8 m) and a strip of 1.4 m x 6 m was mown (pre-cut) within each enclosure because, at that time of year, these plots could generate a large biomass and cutting would probably stimulate grass growth and N uptake. Within the enclosures, one (2010) or two (2011, 2012) strips (1.4 m x 6 m = 8.4 m^2) were mown in mid October. Dry matter yield of the grass was determined and herbage samples were analysed for N content to calculate the N export by the mown grass. Dry matter yield was not determined on the grazed area. At the end of the growing season, around 15 November, one (2010) or two (2011, 2012) strips were cut within the enclosures (8.4 m^2) to estimate the amount of N present in the grass. Within and outside the enclosures, soil was sampled 3 times: at the beginning of the experiment, in mid October at the time of cutting, and in mid November. In 2011 and 2012, soil samples were taken within the enclosures on the two strips, i.e. with and without a pre-cut. The soil sample outside

the enclosures was taken near the enclosure to ensure that this soil had conditions at the beginning of the experiment similar to those within the enclosure. Samples were taken in 3 layers: 0-30 cm, 30-60 cm and 60-90 cm, with 6 drillings (drilling density on the mown strip: 7 drillings per 10 m^2). The sampling methodology was the same for each pasture and each time of sampling. The soil samples were analysed for NO_3-N and NH_4-N according to ISO 14256-2:2005. The nitrogen input by the grazing cattle was calculated by the number of grazing days and excretion figures from VLM (http://www.vlm.be/landtuinbouwers/mestbank/dierlijkeproductie/Berekeningvandenetto-uitscheidingvanrunderen/Pages/default.aspx)

Results and discussion

Grazing cows excrete nitrogen on the pasture in a very heterogeneous way in space and time. The N efficiency is very low, especially during grazing in autumn. This makes it difficult to sample adequately and can help to explain the high variability in NO_3-N and NH_4-N concentrations in the soil between the 3 measurements per treatment, and per sampling period within a pasture. As a result, a pairwise t-test was used on the average of the 3 measurements per pasture in each sampling period to compare cutting without pre-cut with grazing (n=27) or to compare cutting with and without a pre-cut (n=15).

Cutting versus grazing
Please refer to Table 1 for relevant results.

Table 1. Mineral N content in the soil in September (start of experiment), October and November under grazing and cutting (in the enclosures) on permanent grassland in Flanders

| | | | Mineral N-content in the soil profile 0-90 cm | | | | | | | | | | | |
| | | | NO_3-N kg.ha^{-1} | | | | NH_4-N kg.ha^{-1} | | | | NO_3-N + NH_4-N kg.ha^{-1} | | | |
Period	Treatment	Parcels	0-30	30-60	60-90	**0-90**	0-30	30-60	60-90	**0-90**	0-30	30-60	60-90	**0-90**
Sept.	Grazing (G)	27	25	15	9	**49**	13	7	4	**24**	38	22	13	**73**
	1 Cut (C)	27	23	16	9	**48**	13	5	5	**23**	36	21	14	**71**
	P value		*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Oct.	Grazing (G)	27	18	14	12	**43**	26	7	6	**39**	44	21	18	**82**
	1 Cut (C)	27	16	16	16	**47**	14	5	5	**23**	30	21	21	**71**
	P value		*ns*	*ns*	*ns*	***ns***	*0.024**	*ns*	*ns*	*0.049**	*ns*	*ns*	*ns*	***ns***
Nov.	Grazing (G)	27	22	16	12	**50**	21	5	4	**31**	43	21	16	**81**
	1 Cut (C)	27	19	17	15	**51**	12	5	4	**21**	31	22	19	**72**
	P value		*ns*	*ns*	*ns*	*ns*	*0.034**	*ns*	*ns*	*0.038**	*0.029**	*ns*	*ns*	*ns*

*: P< 0.05   ns: not significant

In September, the NO_3-N, NH_4-N and the mineral-N concentration in the soil were the same (no significant differences) inside (cutting) and outside (grazing) the enclosures at the beginning of the experiment for each layer (0-30 cm, 30-60 cm, 60-90 cm, 0-90 cm). The total mineral N content in the 0-90 cm soil layer was on average 72 kg N ha^{-1} and was composed of 49 kg ha^{-1} NO_3-N and 23 kg ha^{-1} NH_4-N. In the middle of October, on the cutting date, the only significant difference ($P<0.05$) was for the NH_4-N concentration in the 0-30 cm and 0-90 cm soil profiles. The NH_4-N concentration in these soil layers was higher for grazing than for cutting. No significant differences in NO_3-N concentration were found between grazing (on average 43 kg ha^{-1} in 0-90 cm soil) and cutting (on average 47 kg ha^{-1} in 0-90 cm soil) for any of the soil layers. In mid-November, at the very end of the grazing season and at the end of the official monitoring period for soil sampling according to the Nitrate Directive (91/676 EU) in Flanders, the only significant differences were found for the NH_4-N content in the 0-30 cm and 0-90 cm soil layers and for the mineral-N content in the 0-30 soil layer. There were no significant differences in the NO_3-N content in any of the soil layers between cutting and grazing. The average values in

the 0-90 cm layer were very close to the data at the beginning of the experiment (September): for grazing in September and November, 50 kg ha^{-1} and 49 kg ha^{-1}, respectively; and for cutting in September and November, 51 kg ha^{-1} and 48 kg ha^{-1}, respectively. For the total mineral-N content in the 0-90 cm layer there was a tendency towards higher N content for grazed pastures in November (81 kg N ha^{-1} versus 73 kg N ha^{-1} in September). A higher NO_3-N concentration in the soil on the grazed area was expected because grazing intensity and consequently deposition of urine (N) on the pasture was high but very variable between the parcels, depending on stocking rate and number of grazing days. The average N deposition by the grazing cattle in the period September-November was 92 kg ha^{-1} ($\pm$ 50 kg N ha^{-1}). Export of N by grazing cattle was not estimated but the N export by cutting was, on average, 75 kg ha^{-1} ($\pm$ 11 kg N ha^{-1}). In the literature, a lower nitrate content in the soil (De Vliegher *et al.*, 2004) or a lower mineral N content in the soil (Holshof and Willems, 2003) have been reported.

A pre-cut or not at the beginning of September?
There were no significant differences between a single cut in October and a cut at the beginning of September followed by a cut in October, for NO_3-N, NH_4-N and the mineral-N content in every soil layer in October, as well as in November. At the end of the growing season, the average NO_3-N content and the mineral-N content in the 0-90 cm layer was 36 kg ha^{-1} and 57 kg ha^{-1} for one cut in October and 50 kg ha^{-1} and 71 kg ha^{-1} for a cut at the beginning of September followed by a cut in October. These differences were not significant, however. Cutting in September and October resulted, on average, in an N export of 99 kg ha^{-1} ($\pm$ 48 kg ha^{-1}) and by cutting only in October, the average N export was 74 kg ha^{-1} ($\pm$ 24 kg ha^{-1}). Cutting twice resulted in an extra N export of 24 kg N ha^{-1} ($\pm$ 30 kg ha^{-1}).

Conclusion

In this experiment, cutting instead of grazing in the period September – November resulted in a significant decrease in NH_4-N content in the 0-30 cm soil layer in October and November and a significant decrease in total mineral-N content in the 0-30 cm soil layer in November. NO_3-N, which is sensitive to leaching, was not significantly affected in this period, which is in contrast with findings reported in the literature. Cutting twice had no significant effects on NO_3-N, NH_4-N and mineral N content in the soil in comparison with a single cut.

Acknowledgements

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Impact of plant diversity, with equal number of grass and legume species, on sward productivity and legume content under contrasted mowing management in a low input system

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Abstract

Studies have underlined the link between plant biodiversity and sward productivity. Higher production is associated with complementarities between the different functional groups involved. More especially, the occurrence of legumes seems to be a key point. In this context, this study aimed to validate the interest of improving species number in the sward on its productivity while controlling for the legume-occurrence effect, in a trial performed under organic farming and mowing schemes (3 and 4 cuts per year). To test the hypothesis, mixtures based on perennial ryegrass-white clover, cocksfoot-lucerne and *Lolium hybridum*–red clover, were diversified through the addition of grass-legume pairs until mixtures with six species of grass and six species of legumes were obtained. The performances of these mixtures (production in quantity and quality and plant composition) were followed during three full exploitation years. As reported previously by Sanderson (2010), our results underlined that there was no unique relationship between herbage yield and the complexity (number of species) of the mixture: everything is a function of the potential of the initial pair of species in the given soil-climate context and of its persistency. Legume content was also crucial, but did not, by itself, explain the performances recorded.

Keywords: functional diversity, mixture persistence, ryegrass-white clover, cocksfoot-lucerne, *Lolium hybridum*-red clover

Introduction

Faced with increased input costs, farmers want to reduce fertilizers and use of external feedstuffs while optimizing internal resources. This evolution in management of farming systems is in response to greater market instability and also to social demand. In such a context and for herbivores production systems, grassland agro-ecosystem productivity, in terms of quantity, quality and stability are key points to insure technico-economical performances and resilience of the farming system. Previous studies have shown the link between plant biodiversity and sward productivity, in terms of quantity (e.g. Hector and Loreau, 2001) and stability (Tilman *et al.*, 2006). Higher production is associated with complementarities between different functional groups involved, complementarities improving resources valorization. The occurrence of legumes seems to be the key point to improve and stabilized sward productivity (Sanderson, 2010).
In this context, the aim of this study was to validate the interest of improving species number in the sward on its productivity while controlling for the legume-occurrence effect. This trial was performed under organic farming and mowing schemes.

Materials and methods

In order to test the impact on grassland production and plant diversity evolution of plant species diversity in mixed grass-legume (60%: 40% of viable seeds) and of management schemes, the following experimental scheme was set up, in a four complete blocks design, in a organic field, converted in 1998, in Libramont, Belgium (49° 55' N 5° 35' E).

Mixtures, based either on perennial ryegrass (*Lolium perenne* L.) - white clover (*Trifolium repens* L.), on cocksfoot (*Dactylis glomerata* L.) – lucerne (*Medicago sativa* L.) and on *Lolium hybridum* – red clover (*Trifolium pratense* L.), were increased in complexity through the addition of grass-legume pairs until mixtures with six species of grass and six species of legumes were obtained (Table 1). Two modalities with, respectively, only grass (12) or legume (10) species were also included in the experimental scheme.

Table 1. Mixtures compared under contrasted cutting management schemes. Timothy (*Phleum pratense* L.); tall fescue (*Festuca arundinacea* Schreb.); meadow fescue (*Festuca pratensis* Huds.); smooth meadow-grass (*Poa pratensis* L.); red fescue (*Festuca rubra* L.); brome (*Bromus sitchensis* Trin); Egyptian clover (*Trifolium alexandrinum* L.); sainfoin (*Onobrychis sativa* Lamarck); yellow trefoil (*Medicago lupulina* L.); subterranean clover (*Trifolium subterraneum* L.); birdsfoot trefoil (*Lotus corniculatus* L.); alsike clover (*Trifolium hybridum* L.).

Mixture basis	Perennial ryegrass /White clover (**PW2**)	Cocksfoot /Lucerne (**CL2**)	*Lolium hybridum* /Red clover (**HR2**)
Four species mixtures	+ Cocksfoot /Red Clover (**PW4**)	+ Tall fescue /Red clover (**CL4**)	+ Perennial ryegrass /White clover (**HR4**)
Eight species mixtures	+ Tall fescue & Timothy grass /Lucerne & Yellow trefoil (**PW8**)	+ Perennial ryegrass & Timothy grass /White clover & Yellow trefoil (**CL8**)	+ Italian ryegrass & Tall fescue /Lucerne & Egyptian Clover (**HR8**)
Twelve species mixtures	+Smooth meadow-grass & Meadow fescue / Bird's-foot trefoil & Alsike (**PW12**)	+Brome & Red fescue /Bird's-foot trefoil & Subterranean clover (**CL12**)	+Timothy grass & Brome /Yellow trefoil & Sainfoin (**HR12**)

The contrasted management schemes applied during three seasons consisted of two cutting schemes reflecting intensive (4 cuts per year – 4C) or extensive (3 cuts per year – 3C) practices (fertilization with 35 t ha^{-1} of composted beef cattle manure).

Before each cut, proportions of grass and legumes species were quantified, on a weight basis, after hand sorting of a composite sample of the four replicates of the corresponding modality.

Before the second cut, the proportions of each species in the mixture were also defined. Due to the difficulties of distinguishing between Italian and Hybrid ryegrasses, these different species were regrouped in an HI group.

Individual plots, measuring 1.5 × 11 m, were harvested using a Haldrup plot harvester. Green forage was weighed and a sample was collected from each plot and dried to quantify dry matter yield and to determine its feeding value (cellulase digestibility).

Data analysis (ANOVA including species richness and block parameters and multiple means comparison based on Student Newman Keuls method) were done using SAS 9.2. software.

Results and discussion

As expected, both the yield (10557±454 *vs* 9490±505 kg DM ha^{-1}) and quality (66.7±1.4 *vs* 70.2±1.6 %), for 3C and 4C respectively, were affected by the number of cuts. Statistical analyses, performed independently for both management schemes, highlighted a significant to highly significant effect of the mixture basis species number × year interaction on DM yields ($F_{3C}(12,105) = 12.3$; $P < 0.001$ and $F_{4C}(12,105) - 2.2$; $P = 0.01$). Table 2 integrates the results of the analyses performed per mixture basis and year.

These results show that once the binary mixture is able to exploit the production potential of this specific soil-climate-management situation (*c*. 12 t DM ha^{-1}) there was no further increase due to additional species: this was the case for HR-based mixtures in 2010 and CL-based mixtures across the three years (Table 2). In contrast, mixture complexity improved the productivity of PW-based mixtures: less aggressive in the sowing year than the HR and CL binary mixtures, across the three years and from less long-lasting HR-based mixtures, already the second and third year of production (Table 2). These trends were equivalent for both cutting frequencies. Sward quality was affected more by mixture basis and cropping year than by species richness (Table 2).

Table 2. Impact of species richness on DM yield (t ha^{-1}) and sward digestibility (%), per year and mixture basis. Means sharing a letter are not significantly different (*P* > 0.05).

		PW-3C		PW-4C		CL-3C		CL-4C		HR-3C		HR-4C	
Year	Nbr Sp	DM	DIG	DM	DIG	DM	DIG	DM	DIG	DM	DIG	DM	DIG
2010	2	8.3b	78a	8.8b	81a	11.5a	66c	9.8a	73	13.1a	72b	10.8a	77
2010	4	11.7a	73bc	10.8a	76b	11.8a	69cb	10.7a	73	11.7b	73b	10.5a	78
2010	8	12.0a	71cd	10.6a	75b	11.6a	71.2b	10.5a	75	11.7b	69c	10.1a	75
2010	12	12.4a	73bc	10.7a	77b	12.1a	70.3b	10.3a	76	12.1b	70c	10.0a	75
2010	12G	5.2c	76ab	5.5c	77b	5.2b	76a	5.5b	78	5.2c	76a	5.5b	77
2010	10L	11.4a	69d	9.9ab	74b	11.4a	69cb	9.9a	74	11.4b	69c	9.9a	74
2011	2	7.5c	72a	7.8c	71a	11.5a	64b	11.0a	65	9.3b	67a	9.5b	69ab
2011	4	9.1b	66b	8.8b	69ab	10.3b	64b	9.8b	68	9.8b	68a	9.4b	69ab
2011	8	9.8b	66bc	9.6a	67ab	10.9ab	65b	10.6a	68	11.2a	64b	10.4a	67ab
2011	12	10.5a	65bc	9.8a	68ab	10.6ab	65b	10.4ab	68	11.1a	66ab	10.4a	66b
2011	12G	5.5d	67b	5.1d	69ab	5.5d	67a	5.1d	69	5.5c	67a	5.1c	69ab
2011	10L	9.3b	64c	8.8b	66b	9.3c	64b	8.8c	66	9.3b	64b	8.8b	66b
2012	2	7.3d	68a	6.8b	72a	11.3a	59b	8.4ab	64	8.6c	66a	8.6a	69
2012	4	9.2b	62b	8.5a	66b	9.9b	62ab	8.0b	65	8.5c	66a	7.8b	69
2012	8	10.2a	63b	8.5a	66b	9.6b	61ab	8.5ab	66	11.0ab	62b	9.0a	66
2012	12	10.4a	63b	8.6b	66b	10.4ab	61ab	8.6a	66	11.6a	62b	9.0a	66
2012	12G	8.1c	63b	6.6b	67b	8.1c	63a	6.6c	67	8.1c	63b	6.6c	67
2012	10L	10.4a	62b	8.7a	67b	10.4ab	62ab	8.7a	66	10.4b	62b	8.7a	66

Even if legume content was significantly correlated to sward productivity (r = 0.6; N = 84), across the different mixtures and years, with an increase of 48 kg DM ha^{-1} year^{-1} per percentage increase in legume proportion, the classifications presented in the Table 2 were not modified by performing covariance analyses with the legume occurrence as co-variable (data not shown) confirming an interest improving species diversity in sward-mixture composition. This applies even without taking into account the legume effect, whether the mixture basis is not able to valorise fully the local productivity potential and/or whether it is not persistent enough. Nevertheless, including more than 8 species in the mixture appeared to be inefficient in the context of this trial.

Conclusions

As reported by Sanderson (2010), there was not a unique relationship between herbage yield and the complexity (number of species) of the mixture: everything is a function of the potential of the initial pair of species in the given soil-climate context and of its persistency. Legume content was also crucial but it did not, by itself, explain the performances recorded.

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The effect of different fodder galega-grass mixtures and nitrogen fertilization on forage yield and chemical composition

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Abstract

Fodder galega (*Galega orientalis* Lam.) is a forage legume that has been grown in Estonia for approximately forty years. Pure galega is known to be a persistent and high-yielding crop rich in nutrients, in particular crude protein (CP). Galega is usually grown in a mixture with grass in order to optimize its nutrient concentration, increase dry matter (DM) yield and improve fermentation properties. There are certain grass species suitable for the mixture. In this study galega mixtures with timothy (cv.Tika), meadow fescue (cv. Arni) and bromegrass (cv. Lincoln) were under investigation in four successive years (2008-2012). Three cuts were carried out during vegetation in 2008, 2009, 2011, 2012 and two cuts in 2010. Nitrogen (N) fertilization rates were N0, N50, N100; this was applied in spring before the first and second cuts. Early season N applications to galega-grass swards can help N-deficiency in the spring. The total dry matter (DM) yield varied from 7.2 to 13.6 t ha. DM yield was dependent on the year, mixture and fertilization level. The CP concentration in the DM varied from 149−229 g kg^{-1}. CP was dependent on the year, mixture and fertilization. High N-fertilization favoured grass growth and reduced the role of galega in the sward. The dry and warm summers favoured the galega growth in the years 2010-2011, but 2012 was rainy.

Keywords: Fodder galega, goat's rue, galega-grass mixtures, forage yield, fertilization.

Introduction

Along with other forage legumes, like lucerne and clovers, goat's rue (fodder galega) has been grown in Estonia for almost forty years. Galega is very persistent with a high yielding ability. Results have shown that the yields can possibly be 8.5 to 10.5 tons of dry matter and 1.7 to 1.8 tons of crude protein (CP) per hectare, with CP concentration of 200-220 g kg^{-1} DM (Raig *et al*., 2001). The nutritive value is the highest when the 1st cut is made at shooting, budding or at the beginning of flowering (Raig *et al.,* 2001). In order to connect the need for nitrogen fertilizer with biologically fixed nitrogen, it is optimal to grow galega in a mixture with grass. Of plant nutrients, nitrogen has the highest effect on yield and quality of forage crop. When choosing grasses for mixtures, the speed of species development, duration and the effect on nutritive value should be considered. Earlier results have shown that growing galega in mixtures with grasses improves the nutritive value and ensiling properties of the forage crop (Lättemäe *et al.,* 2005; 2013). The aim of this investigation was to study the different galega-grass mixtures and N fertilization on DM yield and chemical composition of forage.

Materials and methods

The experimental field was established in 2003 in Saku Estonia (local latitude 57° 25$^{/}$) and the data were collected from 2004. The data from four successive years (2008-2012) were recorded in this study. The trial plots were established on a typical soddy-calcareous soil where the agrochemical indicators were as follows: pH$_{KCl}$ 7.4 (ISO 10390); humus concentration C$_{org}$ 4.1%; concentration of lactate soluble P and K were 97 and 166 mg kg^{-1} respectively. Three galega-grass mixtures were used. The galega variety Gale (*Go*) was sown in binary mixtures with meadow fescue cv. Arni *(Fp)* (10 kg seed ha^{-1}), timothy cv. Tika (*Pp*) (6 kg ha^{-1}) and bromegrass cv. Lincoln (*Bi*) (15 kg ha^{-1}) respectively. The sowing rate of the seed of cv. Gale was 20 kg ha^{-1} in all mixtures. In order to increase competitiveness of grasses and yield of the

first cut, three N fertilization levels were used: N0, N50 and N 100 kg ha^{-1} (April, May I or II decade). The crop was cut by scythe, then weighed and samples were taken for analyses. The botanical composition of the crop was determined prior to sampling. A three-cut system was used during harvest and there were three replicates of the plots of each treatment. All statistical analyses were carried out by using the GLM procedure of SAS.

Results and discussion

The results indicate that galega-grass mixtures ensured high DM yield from since the trial field was established. In 2008-2012 the yields varied from 7.2 to 13.6 t ha^{-1} (Table 1). There were significant differences between the average yields at different N levels and mixtures.

Table 1. The DM yield of fodder galega-grass mixtures in 2008-2012

Mixture	2008			2009			2010			2011			2012		
	N0	N50	N100	N0	N50	N100	N0	N50	N100	N0	N50	N100	N0	N50	N100
Go/Fp	11.6	10.7	11.6	7.6	7.2	7.8	10.4	10	9.4	12.7	13.3	12.4	11.9	11.5	10.3
Go/Pp	10.8	11.3	10.8	8.2	11.4	11.4	11.8	10.1	10.9	11.7	11.5	13	11.6	13.5	13.6
Go/Bi	11.3	12.4	10.9	8.1	10.1	11.4	9.8	9.7	10.7	12	11.4	11.7	10.9	11.0	11.9
Ave-rage	11.2	11.5	11.1	8	9.6	10.2	10.7	9.9	10.3	12.1	12	12.4	11.5	12.0	11.9
	LSD 0.05=0.39			*LSD 0.05 = 0.64*			*LSD 0.05 = 0.45*			*LSD 0.05 = 0.60*			*LSD 0.05 =0.40*		
	**LSD0.05=0.22*			**LSD 0.05 =0.37*			**LSD 0.05 =0.32*			**LSD 0.05 =0.41*			**LSD0.05=0.29*		

*- Least significant difference of N treatment; **- LSD$_{0.05}$ of mixture treatment

The average yield was higher in 2011 and variable, varying from 11.4 to 13.3 t ha^{-1}. There were significant differences between Gale-Arni vs. Gale-Tika and Gale-Lincoln as well as Gale-Tika and Gale-Lincoln at N0 and N100 fertilization levels. Application N fertilizer changed the botanical composition of the sward. N fertilizer increased grasses and reduced the cv. Gale proportion in the pasture. The average cv. Gale proportion in the pasture was 59% in 2008. In the year 2009 the proportion of cv. Gale declined down to 27% due to frost damage during the second decade of May (temperature declined down to -3.9 degrees). When the two-cuts system was applied the Gale pasture recovered in 2010. The Gale proportion also declined considerably when fertilization increased (Figure 1.). The average Gale proportion is essential at N0 and N50 treatments. When it is higher the CP concentration increases in the crop (r=0.53; P<0.05). At fertilization level N0 and N50 the meadow fescue cv. Arni was less competitive. The highest competitiveness was shown by the bromegrass Lincoln at N100 fertilization in 2009.

The nutritive value of mixtures is presented in Table 2. In general, the nutritive value of mixtures was mainly dependent on fertilization level. Lower CP and metabolizable energy (ME) concentrations were found in treatments when N fertilizer was not used. When the fertilization level increased, CP concentration and ME increased but NDF and ADF decreased. Galega usually has a faster rate of development than grasses. Therefore, the ADF and NDF concentrations were higher in treatments where the proportion of cv. Gale was higher and in Gale/Lincoln treatment, due to the higher fibre concentration of the bromegrass.
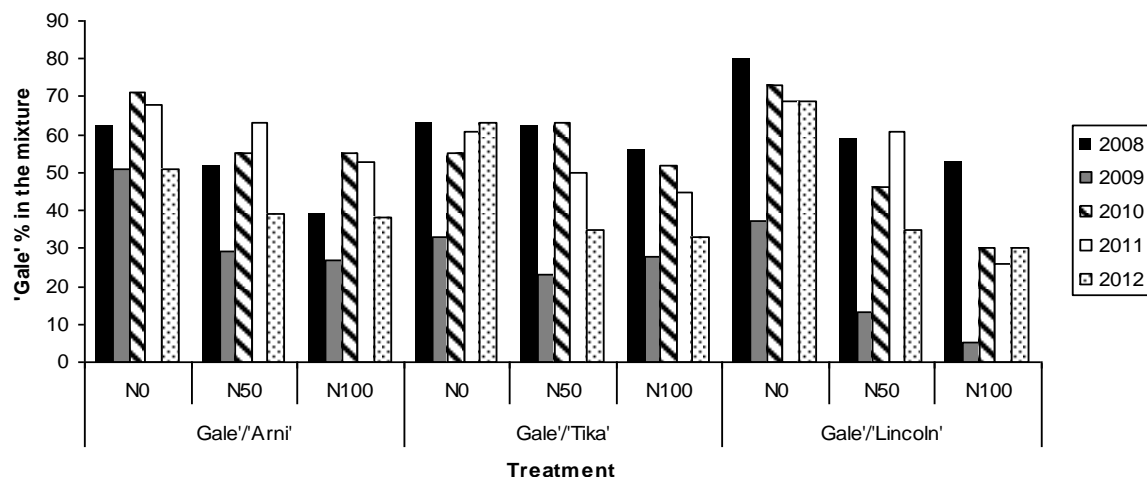
Figure 1. The botanical composition of galega-grass mixture in the first cut in 2008-2012

Table 2. The nutritive value of the fodder galega-grass mixtures of first cut in 2008-2012

Mixture	N fertilizer	CP g kg^{-1} DM	NDF g kg^{-1} DM	ADF g kg^{-1} DM	ME MJ kg^{-1} DM
Gale/Arni	N0	183	466	315	10.1
Gale/Arni	N50	187	459	312	10.2
Gale/Arni	N100	206	413	286	10.5
Gale/Tika	N0	177	486	328	9.9
Gale/Tika	N50	203	450	309	10.2
Gale/Tika	N100	211	435	291	10.5
Gale/Lincoln	N0	165	462	335	9.8
Gale/Lincoln	N50	187	449	327	9.9
Gale/Lincoln	N100	198	488	310	10.2

Conclusions

The galega-grass mixtures maintained high yielding ability and nutritive value for many years. The nutritive value of mixtures was mainly dependent on fertilization. High N fertilization rate favoured grass growth but reduced the role of galega in the sward. The similar higher ME value was obtained in Gale-Arni and Gale-Tika mixtures. The ME concentration was lower in Gale-Lincoln mixture due to higher fibre concentration of bromegrass comparing to other grasses. On the basis of these results, fertilization rate of N50 should be recommended in order to avoid grasses being lost from the pasture and can help N deficiency in the spring.

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Grass only and grass-white clover (*Trifolium repens* L.) swards: herbage production and white clover performance

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Abstract

White clover (*Trifolium repens* L.; Wc) is the most important forage legume in temperate regions of the world. This experiment compared herbage production and tiller density in grass only (GO) and grass-white clover (GWc) swards in 2011 (year (yr) 1) and 2012 (yr 2). Sward Wc content and stolon mass in the GWc swards were quantified. Both swards received 260 kg N/ha/yr. There was no sward type effect on total herbage production in 2011 (14040 kg DM/ha). In 2012, the GWc swards produced more herbage than the GO swards, but this was not statistically different (14740 and 13580 kg DM/ha, respectively). White clover content was not statistically (P=0.12) different between yrs (2011 - 12.6%; 2012 - 21.8%). The perennial ryegrass (*Lolium perenne* L.) tiller density was greater in 2011 than in 2012 (P<0.001) and was greater for the GO than for the GWc swards (5952 and 4934, tillers/m^2 P>0.001). Stolon mass was less in 2011 than 2012 (18.6 and 37.1 g DM/m^2, respectively P>0.01), suggesting that WC becomes more established in the sward in the second production year. Including WC in grass swards can increase herbage mass in the second production year and tiller density declines as stolon mass increases.

Keywords: *Trifolium repens* L., *Lolium perenne* L., white clover content, herbage mass, tiller density

Introduction

White clover (*Trifolium repens* L.; Wc) is the most important forage legume in temperate regions of the world (Frame and Newbould, 1986). When sown in mixed perennial ryegrass (*Lolium perenne* L.; PRG) Wc swards, Wc content is thought to stabilize at 20% of total DM production two years post-sowing when appropriately managed (Andrews *et al.*, 2007). Sward Wc dominance is related to PRG tiller density; Wc content declines as tiller density increases beyond 5000 tiller/m^2 (Brereton, 1995). The objective of this study was to investigate the effect of WC inclusion in a PRG sward on herbage production and tiller density during the first and second production years.

Materials and methods

In May 2010 swards of PRG-only (GO) and PRG-Wc (GWc) were sown at the Dairygold Research Farm, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland. The GO swards were a 50:50 mixture of Astonenergy (tetraploid) and Tyrella (diploid) PRG cultivars sown at 37 kg/ha, and the GWc swards contained the same PRG mix and a 50:50 mixture of Chieftain and Crusader clover cultivars sown at 5 kg/ha. Swards were grazed by rotational stocking with lactating dairy cows from March to October in 2011 and 2012. There were 8 rotations (rot) in 2011 and 11 in 2012. Swards received 260 kg N/ha applied between February and mid-September. Swards were grazed once in late February of each year, before the start of the experiment. Pre-grazing

herbage mass (PGHM) (>4 cm) on a DM basis was determined twice weekly for each sward type (ST) by cutting three strips 1.2 m wide and a known length in the area due to be grazed next and dried at 90ºC for 15hrs to determine DM. Sward Wc content was determined twice weekly; random herbage samples >4 cm were taken across the paddock and separated into grass and Wc fractions and dried at 40ºC for 48 hours to determine the DM proportions. Forty-five turves (10 cm × 10 cm) were removed at random from each ST four times in 2011 and three times in 2012 to estimate PRG tiller density and Wc stolon mass (tiller/m^2 and DM/m^2, respectively). The PRG and other grass tillers (mainly, *Poa annua* L.) were separated and counted. The Wc stolons were removed from each turf, the roots and leaves removed, and then gently washed to remove excess soil; the stolons were then dried at 40 °C for 48 h and weighed to obtain kg DM/ha, as described by (Harris, 1994). Data were averaged as one value/paddock per rot for PGHM and sward Wc content and per collection or month (mo) for tiller density and stolon mass were analysed using PROC MIXED (SAS, 2005). The model included ST, rot or mo, yr, and the associated interactions that at least tended to be significant (*P*<0.1) as fixed effects; and the rot/mo within each yr was used as the repeated measure.

Results and discussion

The PGHM were greater in 2011 than 2012 (1700 and 1400 kg DM/ha SE=445, respectively; *P*<0.01), but were similar between the two treatments across the grazing season. There was no ST effect on total herbage production in 2011 (14040 kg DM/ha). In 2012, the GWc swards produced 1160 kg DM/ha more than the GO swards but were not statistically different (14740 and 13580 kg DM/ha, SE=701, respectively; *P*=0.44). The low sward-Wc content in 2011 is likely to have contributed to the lack of difference between the swards. Andrews *et al.* (2007) showed that HM is increased when sward-Wc content is greater than 20%, which could explain the slight increase in herbage production in 2012. The GWc sward-clover content was low in the first rot (8.8% and 8.4% in 2011 and 2012, respectively) and then increased as time progressed, similar to previous findings (Frame and Newbould, 1986; Brereton, 1995). Annual sward-Wc content was not statistically (*P*=0.12) different between the years (2011 - 12.6% and 2012 - 21.8%; SE=3.35). Although not statistically different, GWc swards had a higher annual clover content in 2012, suggesting that Wc was becoming more established in the sward during the second production year, which agrees with Frame and Newbould (1986) and Brock and Kane (2003). Following this trend, the clover DM yield tended (*P*=0.09) to be greater in 2012 than in 2011 (3170 and 1780 kg DM/ha, respectively, SE=445.8). The PRG tiller density was greater in 2011 than in 2012 (6110 and 4775 tillers/m^2, respectively; *P*<0.01) and was greater for the GO than for the GWc swards (5952 and 4934 tillers/m^2, respectively, *P*<0.01), which would agree with Brereton (1995) who reported that stolon mass would be decreased with a tiller density beyond 5000 tillers/m^2. Harris (1994) also reported that as tiller density decreases, the stolon mass increases, similar to that seen in this experiment. Stolon mass was lower in 2011 than in 2012 (18.6 and 37.1 g DM/m^2, SE=4.6, respectively, *P*<0.01). The lowest value for stolon mass content in the GWc swards was observed in February 2011 (4.9 g/m^2) and stolon mass steady increased in 2011 and remained relatively constant in 2012. The maximum stolon mass value was obtained in October 2012 (47.6 g/m^2; Figure 2). The greater stolon mass and the numerically greater sward-Wc content in 2012 (the second production yr) suggest that Wc stabilization in the mixed swards was on going in the first production yr, which would agree with Harris (1994) and Garwood (1969).
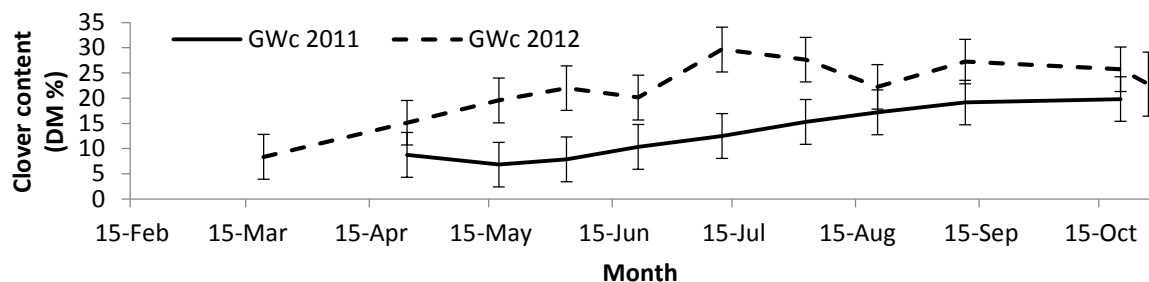
Figure 1. Sward white clover content for 2011 and 2012
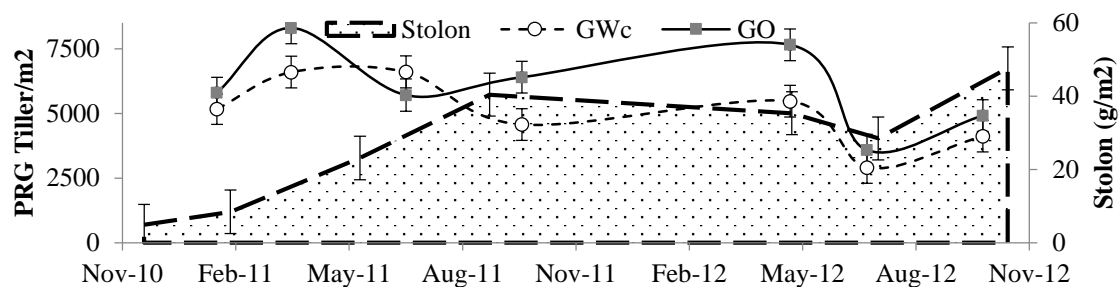
Figure 2. Grass tiller density and stolon mass in 2011 and 2012 in grass-only (GO) and grass-white clover (GWc) swards

Conclusion

When sward-Wc content was low in 2011 there was no difference in herbage production. As sward-Wc content increased in 2012, tiller density in the GWc swards decreased, herbage mass slightly increased and Wc DM yield increased. This shows that as Wc content becomes more established in the sward in the second year, herbage production and Wc DM yield can increase.

Acknowledgements

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The persistence of perennial ryegrass cultivars (*Lolium perenne* L.) in binary mixtures with white clover (*Trifolium repens* L.) under grazing

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Abstract

The persistence of highly productive forage species in pastures is essential to maximize economic returns from grazing livestock. However, most forage cultivars are neither selected nor evaluated under grazing. To test the persistence of ryegrass (*Lolium perenne* L.) cultivars under grazing, a five-year plot trial was conducted on commercial dairy farms located in different climatic conditions in Switzerland. Plots were arranged in a randomized, complete block design with three replicates and sown in autumn 2007 and spring 2008 in binary mixtures with white clover (*Trifolium repens* L). The relative frequency of the perennial ryegrass cultivars was evaluated in 2008, 2009 and 2012 to determine their long-term tolerance to grazing. Significant interactions between both site and cultivar and site and years were found, revealing the importance of the site and its management for the performance of the cultivars. The persistence, as determined by the relative frequencies of the ryegrass cultivars tested, corresponded in the short-term (second and third years) to the rankings of the official variety trials but differed for the long-term observation (in the fifth year). This suggests the need for additional long-term observations under grazing conditions as an extension of the official variety recommendations for cost-efficient, pasture-based livestock production.

Keywords: *Lolium perenne*, grazing tolerance, persistence, cultivar

Introduction

Its tolerance to grazing, its reproductive performance and its excellent nutritional value make perennial ryegrass (*Lolium perenne* L.) the principle grass plant for pasture-based dairy systems in temperate climates (Mosimann, 2002). Persistence is gaining an important role in minimizing costs through reducing the expenses for reseeding while maintaining good productivity of the grass sward. However, most forage cultivars are neither developed nor evaluated under grazing (Brummer and Moore, 2000). This is also the case for the official Swiss variety trials for perennial ryegrass conducted by Agroscope, the Swiss federal research institute for the agro-food industries, in which cultivars are evaluated under a cutting regime only. To simulate pasture conditions, plots for plant-density investigations were cut more frequently (Suter *et al.*, 2012). Specific factors, which may affect the performance of a cultivar under grazing such as trampling, fouling, and tiller pull-up, are not present or may not be as prevalent, as in a clipping situation (Hopkins, 2005). Nevertheless, persistence (evaluated after three years) and the ability to build dense plant populations (stand-density) as an indirect indicator of grazing tolerance are evaluated. To assess tolerance to grazing, we tested seven official recommended cultivars under grazing conditions over a period of five years to compare the correspondence between the official substituted pasture parameters (persistence and stand-density) and the effective performance under grazing pressure.

Materials and methods

Seven officially tested and recommended perennial ryegrass cultivars (Arara, Salamandra, Elgon, Soraya, Alligator, Arvicola, Artesia) (Suter *et al.*, 2012) were sown in field plots (3m × 7m) on five different dairy farms in Switzerland in autumn 2007 and spring 2008 in binary mixtures with white clover (*Trifolium repens* L.). The experimental sites covered a wide range

of climatic conditions and farm management practices (altitude (430 – 600 m a.s.l., precipitation (650 – 1100 mm/year), pasture type/pressure (continuous, rotational stocking), fertilizer use (80-190 kg N, 40-90 kg P, 100-150 kg K, 0-20 kg Mg/ha per year in the form of slurry and/or different mineral fertilizers) and sowing season (autumn 2007, spring 2008). The perennial ryegrass cultivars were sown at seed rates of 15 kg/ha (Arara) and 20 kg/ha (others) in binary mixtures with two white clover cultivars, differing in leaf size, and sown at a seed rate of 25 kg/ha and 15 kg/ha. At each site, the plots were repeated three times in a randomized complete block design. The relative frequency of the cultivars was measured in every plot in the autumn of 2008, 2009 and 2012 according to Daget and Poissonet (1969) by observing the plants at 50 points per plot at an equidistance of 10 cm.

To analyse the effects of the cultivars, sites and time, a Brunner-Langer F2-LD-F1 model for longitudinal data was fitted using the nparLD package in R (R Core Team, 2013).

Results and discussion

Generally, relative frequencies of all cultivars within the experiment strongly declined in 2012 compared to 2008 and 2009 (Table 1). The development of the relative frequencies of the cultivars tested over the five years showed a statistically significant site × year ($P < 0.001$) and site × cultivar ($P < 0.05$) interaction. Both interactions indicate that the individual cultivars responded differently to the site-specific climatic conditions and/or the management practices, which underlines the importance of testing cultivars under a broad range of climatic and management conditions.

The mean relative frequency per cultivar over the five years was highest for Arara and Arvicola (Table 1). This corresponds to the official variety results for stand-density, which also showed higher values for both cultivars (Suter *et al.*, 2012). The official stand-density ranking for Arara as the only diploid cultivar differed connotatively from the ranking of the other cultivars. This may be related to the fact that diploid cultivars generally build more tillers than tetraploids (Laidlaw, 2004). Salamandra and Alligator were the cultivars with low stand densities in the official testing programme. For both cultivars, the lowest relative frequency after 5 years was measured in our experiment. This indicates that the more frequent cutting in the official tests was a reasonable simulation of the short-term grazing effect on given cultivar.

Arara and Arvicola had the highest relative frequency after five years (Table 1).

Table 1. Relative frequencies (as %) of seven perennial ryegrass cultivars over a period of five years at five different sites under grazing conditions.

| Cultivar | Site | | | | | | | | | | | | | | | Mean *(s.d.)* | | |
| | Champvent | | | Gampelen | | | Hessigkofen | | | Hohenrain | | | Waldhof | | | | | |
	08	09	12	08	09	12	08	09	12	08	09	12	08	09	12	08	09	12
Alligator	42	75	34	54	48	27	38	44	26	64	46	29	63	67	31	52 *(12)*	56 *(14)*	29 *(3)*
Arara	42	81	34	78	59	63	39	49	32	73	61	28	65	81	57	59 *(18)*	66 *(14)*	43 *(16)*
Soraya	57	74	35	61	53	48	42	45	29	63	50	43	68	60	39	58 *(10)*	56 *(11)*	39 *(7)*
Artesia	43	73	33	64	57	34	36	44	32	69	58	31	63	69	49	55 *(15)*	60 *(12)*	36 *(8)*
Arvicola	53	79	35	72	60	47	42	50	36	65	50	48	63	71	45	59 *(12)*	62 *(13)*	42 *(6)*
Elgon	51.5	76	33	56	51	28	34	44	34	58	51	36.5	63	62	35	53 *(11)*	57 *(12)*	33 *(3)*
Salamandra	49	58	30	51	53	27	36	36	29	58	50	34	64	69	42	52 *(11)*	53 *(12)*	32 *(6)*

Together with Soraya, these three cultivars showed the least reduction with respect to the relative frequency as compared to 2008 and 2009. We did not find any better persistence for Salamandra and Artesia, in contrast to the official persistence rankings. Alligator was the only cultivar for which we found the expected (according to the ranking of the official variety testing) lower persistence.

The reason for this discrepancy may be explained by differences in the parameters investigated, the different impact of grazing versus cutting conditions or the varying observation period. Nevertheless, it emphasizes the need for long-term observations under grazing conditions to evaluate the persistence of cultivars under grazing conditions.

Conclusion

The results from this study suggest that not only the site, but also the climatic and management conditions are important determinants for the performance of perennial ryegrass cultivars. This implies that cultivars should be tested under a wide range of environmental conditions.Furthermore, although simulating pasture conditions through more frequent cutting, as in the official tests, can result in a fair estimation of the short-term grazing resistance of a cultivar, it is not sufficient for the measurement of long-term grazing persistence, as this study has shown. While evaluating cultivars for cost-efficient, pasture-based livestock production, additional long-term observations under grazing conditions could be a worthwhile extension to the official variety recommendations.

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Grass-only and grass-white clover (*Trifolium repens* L.) swards: dairy cow production

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Abstract

White clover (*Trifolium repens* L.; clover) can increase sustainability of grass-based dairy systems and has the potential to increase milk production. This experiment examined the seasonal effect of grass-white clover (GWc) and grass-only (GO) swards during the first (2011) and second (2012) production year on dairy cow production. Two groups of cows were allocated to graze each sward in 2011 (n=15) and 2012 (n=20). Swards were rotationally grazed. Clover content in GWc swards tended to be less in 2011 than 2012 (18.0 and 25.3 dry matter (DM)%, respectively, P=0.08). Cows grazing both swards had similar total milk and milk solid yields (2011: 3494 and 271; 2012: 4242 and 341 kg/yr, respectively). However, in 2012, GWc cows had slightly higher milk production than the GO cows from June onwards, a response to the greater sward clover content in those months. Sward type had little effect on milk fat, protein and lactose content, 4.52, 3.65 and 4.57%, respectively. It is concluded that the potential of clover to increase milk production depends on sward clover content.

Keywords: *Trifolium repens* L., *Lolium perenne* L., mixed sward, dairy cow, milk production

Introduction

White clover (*Trifolium repens* L.; clover) can increase the sustainability of grass-based dairy systems (Frame and Newbould, 1986). Clover has better nutritional quality than perennial ryegrass (*Lolium perenne* L.; grass), and grass-clover swards (GWc) can increase dairy cow voluntary dry matter (DM) intake (DMI) compared to grass-only swards (GO), and cows grazing GWc can produce more milk (Harris *et al*., 1997). However, clover growth is dependent on temperature and therefore clover content in GWc swards varies throughout the year (yr). The objective of this experiment was to assess the seasonal effect of clover inclusion in grass swards during the first (2011) and second (2012) production years on dairy cow production.

Materials and methods

Grass-only and GWc swards were sown at Dairygold Research Farm, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland in May 2010 and both received 260 kg N/ha/yr. Thirty/40 spring-calving dairy cows were allocated to graze each sward type (ST) from April/March to October of 2011 and 2012, respectively. Swards were rotationally stocked and fresh herbage was offered daily (16 and 17 kg DM/cow/d in 2011 and 2012, respectively, at 4 cm above ground level) after morning milking, and 1 kg concentrate/cow/d in 2011. Concentrate supplementation was only offered in 2012 when feed demand exceeded grass supply. A herbage sample from the GWc sward was separated into grass and clover components to estimate sward clover content on a DM basis twice-weekly. Daily milk yield (MY) was recorded at each milking (7:30 h and 15:30 h; Dairymaster, Causeway, Co. Kerry, Ireland). Milk composition (fat (MFatc), protein (MProtc) and lactose (MLacc)) was determined weekly. Milk solids yield (MSY) was

calculated as the sum of milk fat and protein yields. Cow data were averaged as one value per cow/month (mo) and as one clover content value per paddock/mo and analysed using PROC MIXED (SAS, 2005). Cow data model included ST, mo, yr, and their interactions as fixed effects; parity, calving date, with the three-week pre-experimental milk data as covariates and the mo as a repeated measure. Interactions and covariates were removed from the model if they did not tend to be significant (P>0.1).

Results and discussion

There was a mo×yr interaction for the GWc sward clover content (Figure 1).
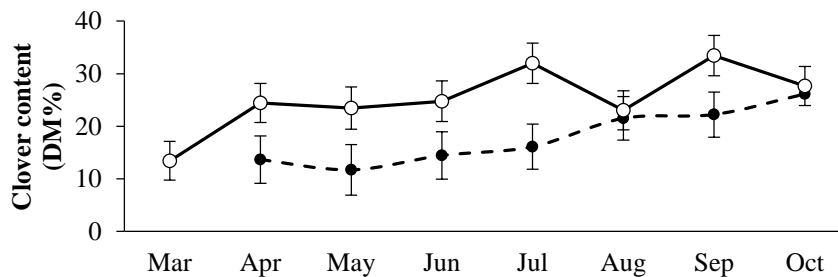
Figure 1. Seasonal clover content in the GWc swards during the first (2011: dashed lines) and second (2012: solid lines) sward production year.

Clover content followed the expected seasonal pattern, with low values in spring and maximum in late summer/early autumn (Frame and Newbould, 1986). Clover content was greater in July (P<0.05) and tended to be greater (P<0.1) in April, May and September in 2012 than in 2011. Annual clover content tended to be greater in 2012 than in 2011 (25.3 and 18.0% of DM, P=0.08). There was a ST×mo×yr interaction for all milk parameters analysed, except for MLacc which only had a ST×mo interaction (Figure 2). MY, MSY and MLacc decreased, but MFatc and MProtc increased across both years. Milk yield and MSY were greater in 2011 than in 2012 from May until August, and were greater for October in 2012. Clover inclusion had no effect on seasonal or cumulative MY or MSY (3494 and 271 kg/yr, respectively) in 2011. In 2012 MY tended (P=0.06) to be greater for GWc cows in June compared to the GO cows and was 19% greater (P<0.05) in August than for GO cows. The GWc cows also had greater (P<0.05) MSY than the GO cows in June, August and October in 2012. However, there was no ST effect on cumulative values (MY 4242 and MSY 341 kg/yr) in 2012. The year differences (P<0.001) in cumulative MY and MSY are related with the longer evaluation period in 2012. Previous work did not find differences in milk production between cows grazing either ST (Phillips *et al.*, 2000; Schils *et al.*, 2000). Such a lack of effect is related to the low clover content observed in the GWc swards. It has been proposed that milk production in mixed swards can be increased when clover content is greater than 30% (Harris *et al.*, 1997). This also explains the MSY differences observed in the second half of 2012. Additionally, under this system cows had a restricted herbage allocation which did not allow the potential benefit in voluntary DMI that clover might have on cow production to manifest (Harris *et al.*, 1997). MFatc was greater (P<0.05) in 2012 than in 2011 in April, May and July and August. MProtc tended (P=0.06) to be greater in April 2012 and also was greater (P<0.05) in May and June 2012 compared to those months in 2011. The GWc cows tended (P=0.06) to have greater MFatc than GO cows in October 2011. Clover inclusion increased the MLacc in September by 2% (4.37 and 4.46%, P<0.05) in both years. There was no significant ST effect but year tended (P=0.1) to affect total MFatc (2011: 4.38% and 2012: 4.67%) and total MLactc (2011: 4.60% and 2012: 4.55%), and had no effect on total MProtc (3.65%). Similarly, other research (Leach *et al.*, 2000; Phillips *et al.*, 2000) did not find a ST effect on milk composition.
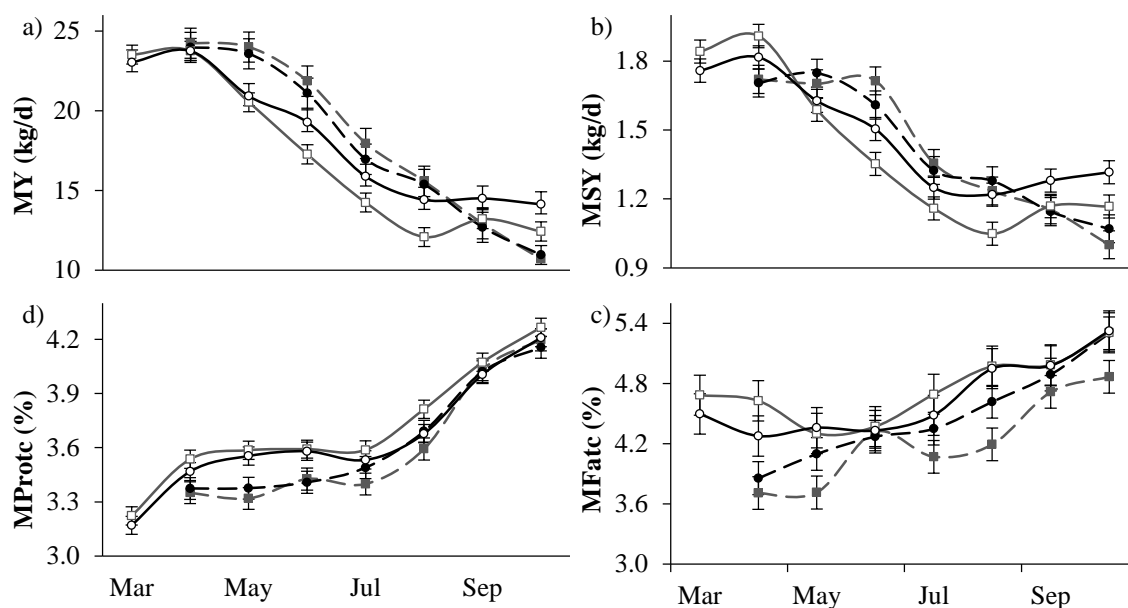
Figure 2. Seasonal effect of ST (GO: squares, GWc: circle) during the first (2011: dashed lines) and second (2012: solid lines) sward production year on dairy cow a) milk yield (MY), b) milk fat % (MFatc), c) milk solids yield (MSY) and d) milk protein % (MProtc).

Conclusion

White clover content in mixed swards was low during spring and greatest in late summer or autumn. White clover inclusion into grass swards had only minimal effect on milk production and composition in the first production year, but increased milk and milk-solids production in the second part of the second grazing season. It is concluded that the potential of white clover to increase milk production depends on sward clover content.

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Effect of grass-only compared to grass-white clover swards on cow rumen function and methane emissions

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Abstract

A study was undertaken to identify the effect of white clover inclusion (*Trifolium repens* L.) in perennial ryegrass swards on dairy cow rumen characteristics and methane emissions. Rumen volatile fatty acids (VFAs), ammonia content and pH of cows grazing grass-only (GO) or grass-white clover (GWc) swards during spring, summer and autumn were assessed. Methane emissions from 20 cows grazing each sward were assessed in autumn. The clover content of the GWc swards was 7.5, 8.8 and 30.9% in spring, summer and autumn, respectively. Clover inclusion influenced rumen characteristics (altered VFA composition, increased ammonia content and rumen pH) with increased effect as the season progressed due to greater sward clover content. Clover effects on rumen characteristics were greater in the afternoon than in the morning due to a greater clover inclusion in the diet during morning grazing. Despite the high sward clover content in autumn, clover inclusion only increased milk fat content; it had no effect on milk protein content and little effect on milk production. Clover inclusion reduced CH_4 emissions per unit of intake but did not affect CH_4 emissions per day or per unit product.

Keywords: *Trifolium repens* L., *Lolium perenne* L., rumen function, methane emission

Introduction

The ability of white clover (*Trifolium repens* L.; clover) to fix N, combined with having a greater nutritional quality than perennial ryegrass (*Lolium perenne* L.; ryegrass), can contribute to improved sustainability of grass-based dairy systems (Frame and Newbold, 1986). Clover content in grass-clover swards (GWc) varies during the grazing season due to temperature and natural growth pattern. Grazing cattle show a partial preference for clover compared to ryegrass (Rutter *et al.*, 2004). It is likely that seasonal clover content and the preference of cattle for clover might interact and alter rumen function and enteric methane emissions (e-CH_4). The objective of this experiment was to identify the seasonal effect of including or not including clover in ryegrass swards on dairy cow rumen function and e-CH_4.

Materials and methods

A grass-only (GO) and GWc sward were sown at Dairygold Research Farm, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland in May 2010. In 2011, 30 spring-calving dairy cows were allocated to each sward type (ST; n=15) from April to October. Swards were rotationally stocked and fresh herbage offered daily (17 kg DM/cow/d) after morning milking. Pre-grazing herbage mass (HM) was estimated twice-weekly using a lawn mower (Etesia UK. Ltd., Warwick, UK). The GWc sward-clover content was estimated twice-weekly. Eight rumen-fistulated dairy cows were arranged into four 2 (treatments) × 2 (14 d periods) Latin squares. This was repeated during SPR (16 May - 10 Jun), SUM (11 Jul - 5 Aug) and AUT (22 Aug - 18 Sep). Rumen samples from these cows were taken after every milking (time of day: T; ~09:00 and ~16:00 h) on the 11th and 12th d of each period, and pH, volatile fatty acids (VFA) and ammonia content were measured. In AUT, five cows were added to each treatment (n=20) to estimate DM intake (DMI) and e-CH_4 using the *n*-alkane (Mayes *et al.*, 1986) and the SF_6

(Zimmerman, 1993) techniques, respectively. Data were averaged as one value/cow/time point/period for the rumen sample data, and as one value/cow for e-CH$_4$ data, and analysed using PROC MIX (SAS, 2005) with cow as a random factor. The e-CH$_4$ data model included the effect of ST and the rumen data model included ST, period, T and the ST×T interaction.

Results and discussion

Pre-grazing HM was similar for each treatment in SPR and SUM (1600±65 and 1680±70 kg DM/ha, respectively), but was greater for GWc than GO in AUT (1880 and 1670±60 kg DM/ha, respectively). GWc sward-clover content was 7.5, 8.8 and 30.9% in SPR, SUM and AUT, respectively. There was a ST×T interaction for some rumen parameters (Table 1).

Table 1. Effect of sward type (ST; GO: grass-only, GWc: grass-white clover), time of day and season on rumen volatile fatty acids (VFA) and pH of rumen-cannulated dairy cows.

		Spring (16 May to 10 Jun)					Summer (11 Jul to 5 Aug)					Autumn (22 Aug to 18 Sep)				
	Time	GO	GWc	sem	ST	ST×T	GO	GWc	sem	ST	ST×T	GO	GWc	sem	ST	ST×T
Total VFA	09:00h	117	119	3.9	ns	ns	109[ab]	104[a]	4.0	ns	*	122	117	3.3	ns	ns
(mmol/L)	16:00h	137	146	3.9			115[b]	129[c]	4.0			158	158	3.3		
Acetic acid	09:00h	63.3[c]	65.3[d]	0.78	ns	***	67.4	67.1	0.61	ns	ns	66.2	67.2	0.45	*	ns
(%)	16:00h	58.2[b]	54.6[a]	0.78			61.8	62.6	0.61			59.3	60.0	0.45		
Propionic acid	09:00h	20.2[b]	18.9[a]	0.60	ns	*	16.2	16.7	0.58	ns	ns	17.2	16.3	0.43	*	ns
(%)	16:00h	22.3[c]	22.5[c]	0.60			18.5	18.9	0.58			21.0	20.5	0.43		
Butyric acid	09:00h	11.8[a]	11.3[a]	0.37	0.07	**	12.7	12.4	0.46	*	ns	13.0	12.7	0.28	**	ns
(%)	16:00h	15.6[b]	17.7[c]	0.37			15.4	14.2	0.46			15.5	14.6	0.28		
Valeric acid	09:00h	1.5[a]	1.4[a]	0.15	ns	*	1.1	1.2	0.04	ns	ns	1.3[a]	1.2[a]	0.05	ns	*
(%)	16:00h	1.8[b]	2.1[c]	0.15			1.5	1.5	0.04			1.6[b]	1.7[b]	0.05		
Iso acids[1]	09:00h	3.2	3.2	0.15	ns	ns	2.6	2.7	0.16	ns	ns	2.3	2.5	0.14	***	ns
(%)	16:00h	2.9	3.2	0.15			2.8	2.9	0.16			2.6	3.2	0.14		
Lactic acid	09:00h	3.5	4.5	0.82	ns	0.07	4.9	4.9	0.81	ns	ns	7.4	10.2	0.57	***	ns
(mmol/L)	16:00h	3.6	2.61	0.82			4.7	4.24	0.84			3.4	4.72	0.57		
Ammonia	09:00h	6.0	5.9	1.87	ns	ns	11.2[a]	11.3[a]	1.11	*	*	6.5[a]	9.1[b]	1.03	***	**
(mmol/L)	16:00h	13.2	16.1	1.87	.		17.6[b]	20.7[c]	1.11			20.3[c]	28.5[d]	1.03		
Rumen pH	09:00h	5.86	5.91	0.075	ns	ns	5.52	5.66	0.120	ns	ns	5.29	5.37	0.069	**	ns
	16:00h	5.30	5.21	0.075			5.99	6.13	0.120			5.81	6.06	0.069		

[1]Isobutyric and isovaleric acids; Times with different superscript letters differ ($P < 0.05$).
† = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

During SPR, clover inclusion increased acetic acid percentage (A%) in the morning, but lowered acetic A% in the afternoon. GWc cows had lower propionic A% in the morning than GO cows but similar in the afternoon. Clover inclusion increased butyric and valeric A% in the afternoon but had no effect in the morning. During SUM, clover inclusion increased total VFA and ammonia contents in the afternoon but not in the morning. There was a ST effect on butyric A% as GWc cows always had a lower butyric A% than GO cows. During AUT, clover inclusion increased ammonia concentration in morning and afternoon, but the difference between the ST was greater in the afternoon. There was a ST effect on a number of the rumen parameters in AUT. GWc cows had a greater acetic, lactic and iso A% (isobutyric plus isovaleric), but lower propionic and butyric A% than GO cows. The acetic and propionic A% responses to clover inclusion were similar to those reported by Ribeiro Filho *et al.* (2012) and were more evident when clover content was greatest (AUT). It was expected that clover inclusion would increase VFA; however, this was only evident in SUM afternoon samples. Iso-acids and ammonia contents responses may be related to the expected high dietary crude protein content of the GWc swards. Clover rumen fermentation effects were more evident in the afternoon, which may be related to GWc cows including clover in greater proportions in their diet in the morning grazing, as cows prefer clover compared to ryegrass (Rutter *et al.*, 2004). Clover inclusion in swards had no effect on rumen pH during SPR and SUM but increased rumen pH in AUT, which was different to the findings of Ribeiro Filho *et al.* (2012). However, an *in vitro* study (Niderkorn

et al., 2011) found a higher pH when a binary mix of ryegrass and clover was incubated for 24 h, compared to ryegrass. Treatments had similar pre-grazing HM during the e-CH_4 measurement week (1970±70 kg DM/ha) and GWc clover content was 24%. Clover inclusion had minimal effect on milk production or its composition (Table 2).

Table 2. Effect of sward type (GO: grass-only, GWc: grass-white clover) on dairy cow performance and methane emissions during autumn (11 to 18 September).

	Cow production				Methane emissions ratio (g CH_4)				
	GO	GWc	sem	P-value		GO	GWc	sem	P-value
					g/cow/d	360	354	13.6	ns
DMI (kg DM/cow/d)	15.0	16.5	0.6	0.07	g/kg of DMI	24.5	21.5	0.84	<0.05
Milk yield (kg/d)	14.1	14.0	0.64	ns	g/kg of milk	26.5	26.0	1.14	ns
MS yield (kg/d)	1.14	1.19	0.047	ns	g/kg of MS	318	306	11.5	ns
Fat (%)	4.38	4.72	0.097	<0.05	g/kg of milk fat	594	558	21.4	ns
Protein (%)	3.76	3.87	0.059	ns	g/kg of milk protein	690	680	27.4	ns

DMI: dry matter intake; MS: milk solids.

The lower milk fat content of the GO cows is in agreement with their lower rumen pH. There was no effect of treatment on e-CH_4/cow/d. However, clover inclusion reduced the e-CH_4 per kg DMI by 12%, comparable to the 9.6% reported by Lee *et al.* (2004). Similarly, those authors did not find differences in e-CH_4 per unit output.

Conclusion

The mixture of ryegrass and clover influenced rumen characteristics with increased effect as the season progressed. The rumen fermentation effects of clover were more evident in the afternoon samples due a greater clover inclusion in the diet in the morning period. However, clover inclusion had little effect on milk production and only increased milk fat content. The presence of clover reduced e-CH_4/kg DMI but did not affect the e-CH_4 per unit output.

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Animal choice for grass-based systems

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Abstract

Grass-based milk production systems require robust and 'easy care' cows capable of high levels of performance from grazed pasture. Cows intensively selected for milk yield are not well suited to seasonal grassland-based systems because of undesirable side effects on reproduction and survival. Dual-purpose or cross-breed cows should be more flexible and better adapted to grassland-based systems. The objective of the research reported in this paper was to compare the suitability of dairy cow breeds for pasture-based systems in France [Holstein (Ho) and Normande (No)] and in Ireland [Holstein-Friesian (HF) (and HF strains), Norwegian Red (NR) and HF×NR] in terms of milk production. In France, the high genetic-merit Ho cow is not suited for systems based on grassland and low concentrate input, whereas the No dual-purpose breed still performed well in these conditions and had higher fertility and milk value. In Ireland, crossing the HF with NR can increase overall animal performance by increasing herd health, fertility and milk value. Overall, the Ho/HF appears to be less flexible and poorly adapted to low-input systems based on the maximization of grassland use for milk production.

Keywords: Dual purpose, crossbreeding, dairy cow, grass-based system, milk production

Introduction

The animal required for efficient grassland-based production systems must be robust and 'easy care', as well as being capable of high levels of performance from grazed pasture. Until recently most experimental results have indicated little or no importance of breed or strain by feeding-system interaction. However, recent studies have shown large differences in performance (especially fertility and survival) and overall farm profitability between diverse breeds and strains of dairy cows (Dillon *et al.*, 2007). Cows intensively selected for milk yield are not well suited to seasonal grassland-based systems because of undesirable side effects on reproduction and survival. Dual-purpose or cross-breed cows should be more flexible and better adapted to grassland-based systems. Dual-purpose dairy-breeds improve milk composition and increase beef value. Crossing the Holstein-Friesian (HF) with an alternative dairy breed sire can increase overall animal performances by increasing herd health, fertility and milk value. The objective of the research reported in this paper was to compare the suitability of dairy cow breeds for pasture-based systems in terms of milk production and fertility.

Materials and methods

An experiment was undertaken at the INRA experimental farm of Le Pin-au-Haras in the North-west of France in Normandy (48.44ºN, 0.09ºE) comparing the Normande (No), a dual-purpose breed, with Holstein (Ho). From 2006, Ho (n=122) and No (n=112) dairy cows differing in genetic potential (evaluated within breed) either on milk potential (High Milk yield group – MY genetic group) or on milk fat and protein content potential (High Milk Solids content group – MSc genetic group), were allocated to two contrasting feeding strategies: the animal adapts itself to the feed available (Low) or the feed available is adapted in a way to satisfy the animal requirement and to allow it to express its genetic potential (High). The breeding period was 13 weeks and cows calved from January to March.

In 2003, a farm participatory study was established in Ireland to enhance breeding value estimation for the Norwegian Red (NR) breed and NR × HF crossbreds. The study was a contemporary comparison design, whereby both parent breeds (NR and HF) and crossbreds (NR×HF) would be present on each farm to provide data relevant to breed and heterosis estimation. Semen from 10 proven NR AI sires was distributed to 55 commercial dairy herds to generate NR×HF crossbred females. In 2004, 393 purebred NR heifer calves sired by the same 10 proven NR AI sires used to generate the NR×HF animals were imported to Ireland. Animal performance data subsequently became available from 46 of these herds. All herds were milk recorded for five lactations and body weight and body condition score (BCS) were recorded. In order to augment the participatory data, herds containing both HF and NR genetics were identified from the national database. Because Ho and Friesian are considered different breeds within Irish genetic evaluations, and due to the intertwined nature of the two breeds within the cow population, it was considered appropriate to examine the relative breed and heterosis effect among the three breeds. Friesian genetics was further categorized as New Zealand Friesian (KF) and British (or European) Friesian (BF). Data from 2004 to 2010 was obtained for all herds. The production file used in routine genetic evaluations containing 305 d milk yields was provided by the Irish Cattle Breeding Federation (ICBF).

Results and discussion

France

The Low feeding strategy resulted in a large reduction in total milk yield (-2000 kg/cow) and milk solids (MS) yield per cow (-70 kg and -72 kg of fat and protein/cow) (Table 1).

Table 1. Impact of the breed (Holstein or Normande), the genetic group and the feeding strategy on the lactating and reproductive performance of the dairy cow.

Breed	Holstein				Normande				Effect (P<)	
Feeding strategy	High		Low		High		Low		Breed	Feed strat.
Genetic group (GG)	MY	MSc	MY	MSc	MY	MSc	MY	MSc		
Milk yield (kg)	8841	8189	6368	5675	6608	6058	5010	4586	0.001	0.001
Fat content (g/kg)	35.8	40.2	36.9	42.1	39.6	42.5	40.1	43.4	0.001	0.001
Protein content (g/kg)	31.2	32.9	30.2	31.8	34.3	35.5	32.6	34	0.001	0.001
Milk Solids (fat +protein - kg)	587	587	421	414	476	457	356	346	0.001	0.001
Conceived (%)	84		61		86		82		0.01	0.01
Recalving (%)	59		44		71		68		0.01	0.01

The response to feeding strategy was greater (*P*<0.001) for Ho cows (-2500 kg milk yield and -168 kg MS) than for No cows (-1500 kg milk yield and -117 kg MS). The No cows produced milk with + 3.0 and + 2.3 g/kg for milk fat and protein content, respectively, in the High and Low feeding strategy. For both breeds, the High feeding strategy reduced milk fat content (-1.5 and -0.8g/kg for Ho and No cows, respectively) and increased protein content (+1 and +1.6g/kg for Ho and No cows, respectively). The phenotypic expression of the genetic group for milk yield, fat and protein content were in agreement with the milk and MS content genetic index and did not have any interaction with feeding strategy. The reproductive performances were generally highly altered for the Ho cow with very low gestation rate especially in the Low feeding strategy. In contrast the No cow does not seem to be sensitive to feeding strategy.

Ireland

The Ho expressed a higher propensity for milk volume compared with the other breed groups investigated (Table 2).

Table 2. Breed and heterosis estimates for 305 d yields of milk, fat and protein. [1]BF=British Friesian, KF=Kiwi Friesian, HO=Holstein, NR=Norwegian Red; [2]SE=standard error

Breed[1]	BF	KF	NR	BF×HO	KF×HO	NR×HO
Milk (kg)	-597	-366	-504	122	249	161
SE[2]	6.3	27.6	26.0	4.7	18.8	24.8
Fat (kg)	-20.6	3.3	-17.9	5.5	19.2	6.4
SE	0.25	1.08	1.02	0.18	0.74	0.97
Protein (kg)	-18.3	-3.3	-14.7	4.4	11.4	6.3
SE	0.20	0.87	0.82	0.15	0.60	0.79

Similarly, breed differences for fat and protein yields were lower for all three compared with the Ho. Although NR milk on average had higher fat and protein content compared to Ho, they produced lower 305-d yields of fat and protein. The data indicate that NR×HF dairy cows are capable of production levels per cow comparable to HF on low-cost systems, but fertility and survival levels are markedly improved, e.g. six-week in-calf rates were increased by over 10 percentage units with NR×HF. Body condition score, a trait genetically associated with differences in reproductive efficiency (Berry, 2003), was higher ($P<0.001$) for the NR at 3.03 compared with the HF at 2.85. That of the NR×HF (2.98) cows was similar to the Norwegian Red but higher ($P<0.001$) than the HF.

Conclusion

The most profitable breed is the one that returns the highest profit per unit of the most limiting input. In France, with contrasted feeding strategies, the Ho breed is much more sensitive compared to the No breed. The high genetic-merit Ho cow is not suited for systems based on grassland and no concentrate input, whereas the No dual-purpose breed still performed well in these conditions and had high fertility and milk value. In Irish grass-based systems crossing the Ho/HF with an alternative dairy breed sire such as NR can increase overall animal performance by increasing herd health, fertility and milk value. Overall, the Ho/HF appears to be less flexible and poorly adapted to low-input systems based on the maximization of grassland use for milk production.

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Effect of sheep breed on lamb production from lowland pasture under continuous stocking

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Abstract

The aim of this investigation was to evaluate the efficiency of lamb production depending on different breeds under grazing by continuous stocking on lowland pasture. The experiments were carried out in 2011-2013 on semi-natural lowland pasture. In the study, four different sheep breeds (4-4.5 month old) were included: 1) White-headed meat sheep, 2) Wielkopolska sheep, 3) Romanov sheep and 4) Blanc du Massif Central sheep. Forage from the pasture was the only feed the lambs received. It can be concluded that Wielkopolska sheep and white-headed meat sheep are suitable for efficient lamb production on lowland pasture in Poland. As a native breed the Romanov sheep is better for utilization of low quality pasture and can play an important role in environmental protection and management of the agricultural landscape, whereas Wielkopolska sheep prefer a good yielding sward under continuous stocking with intensive regrowth during favourable weather conditions for efficient lamb production. Blanc du Massif Central sheep did not appear well adapted for grazing conditions in Poland.

Keywords: breed, continuous stocking, lamb production

Introduction

From the beginning of the last decade of the 20th century in many European countries there has been a decline in the sheep population. In Poland the total population of sheep in 2011 reached about 212.7 thousand heads and total ewes number about 143.8 thousand heads (Goliński, 2012). Sheep, as a grazing animal, are very important for maintaining the multi-functionality of grassland. Therefore, research work concerning the sheep characteristics and management to increase environmental benefits and competitiveness of grassland-based systems are necessary (Niżnikowski *et al.*, 2010; Goliński, 2012). The aim of this study was to evaluate the efficiency of lamb production, for different breeds, under grazing by continuous stocking on lowland pastures.

Materials and methods

The pasture experiment was established in spring 2011 and was completed in October 2013. The experiment took place on semi-natural lowland pasture at the Brody Experimental Station of the Poznan University of Life Sciences (52° 26N, 16° 18E; 92.0 m a.s.l.; long-term mean annual rainfall 601 mm, and mean air temperature 8.3 °C) on a mineral soil, fertilized at the level 50 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1} and 60 kg K_2O ha^{-1} applied in spring. In April 2011 the experimental area was fenced. Four paddocks (P1-P4) were prepared, each of 1000 m^2, for continuous stocking of five lambs per different breed during the vegetative period. In the experiment the following sheep genotypes (4-4.5 month old) were included: P1) White-headed meat sheep, P2) Wielkopolska sheep, P3) Romanov sheep, P4) Blanc du Massif Central sheep. The grazing seasons in 2011, 2012 and 2013 lasted 160 days from 30 April to 7 October. Forage from the pasture was the only feed the lambs received. The animals were on the pasture during the day and night, and fresh water and minerals were available. The sward height was maintained at a constant level of 5-7 cm by changing the stocking rate. A rising plate meter was used to measure sward height. On each paddock, ungrazed plots of 3 m^2 were established to determine sward botanical composition and pasture yield. Sward yield was influenced by

weather conditions during the grazing period (April to September, respectively) characterized by following amounts of monthly rainfall (mm) in 2011: 14.0, 34.0, 52.6, 175.4, 34.5, 46.0, and air temperature (monthly means, °C): 11.7, 14.1, 18.6, 17.9, 18.8, 15.3. In 2012 the rainfall reached following amounts: 22.0, 77.2, 163.0, 197.6, 60.1, 30.0 mm, and air temperatures (as monthly means) were: 8.8, 14.8, 16.0, 19.2, 18.7, 14.3 °C. In 2013 the rainfall reached following amounts: 15.4, 69.8, 125.3, 67.3, 51.5, 33.7 mm and monthly mean air temperatures: 8.0, 14.4, 17.3, 20.1, 19.1, 12.9 °C.

The daily liveweight gain of the lambs was measured by systematically weighing animals at the end of each month (May-September). Sward botanical composition was evaluated using the point method of Levy and Madden (1933). On the ungrazed plots (1.5 m × 2 m) sward yield was determined each month. Tests of the main effects were performed by F-test. Means were separated by LSD and were declared different at $P < 0.05$.

Results and discussion

Botanical composition of the pasture sward was similar on each paddock and was dominated by grasses, particularly by *Lolium perenne*. In the sward, high proportions of *Festuca rubra*, *Phleum pratense* and *Festuca arundinacea* were also determined. *Trifolium repens* occupied only 1.4-1.8%. From the group of herbs *Taraxacum officinale* occurred in quantities of 1.3-2.2%. The total number of species in the pasture sward ranged from 24 to 25, depending on the paddock. It could be concluded that the botanical composition had no significant impact of feed value of the grazed sward by different lamb breeds.

The DM yield of pasture showed significant variations between grazing months and years. Pasture yield was affected by weather conditions. The highest sward yield (1.8 t ha⁻¹ DM) in 2011 was recorded in August, which can be attributed to very good weather conditions for the vegetation. In 2012 the highest sward DM yield was determined in May and reached, on average, 2.6 t ha⁻¹ DM. In this period a large area of pasture was cut and the sward was conserved as hay. In the following months sward yield on the pasture was significantly lower. The lowest DM yield of pasture in the 3-year study period was determined in 2013.

The results presented in Table 1 show that Romanov sheep breed had the highest daily liveweight gain during the grazing season in 2011.

Table 1. Liveweight gain of lambs during the grazing period in 2011-2013 (g day⁻¹)

Sheep breed	Period of lamb gains (month)					Means
	May	June	July	August	September	
			2011			
White-headed meat sheep	135.8	72.6	68.3	84.3	73.6	86.9b
Wielkopolska sheep	84.2	81.7	102.8	114.0	81.6	92.9ab
Romanov sheep	197.9	97.7	72.6	77.9	73.2	103.9a
Blanc du Massif Central sheep	113.7	91.5	101.5	86.1	28.0	84.2b
LSD$_{0.05}$	-	-	-	-	-	11.34
			2012			
White-headed meat sheep	121.8	102.9	116.1	93.4	155.3	117.9a
Wielkopolska sheep	155.2	82.3	106.5	101.4	165.8	122.2a
Romanov sheep	117.0	74.0	97.5	94.0	147.5	106.0b
Blanc du Massif Central sheep	108.5	68.6	101.6	83.6	125.7	97.6b
LSD$_{0.05}$	-	-	-	-	-	8.57
			2013			
White-headed meat sheep	64.0	81.3	73.9	78.2	65.1	72.5
Wielkopolska sheep	92.2	83.2	60.5	71.7	65.4	74.6
Romanov sheep	82.2	68.4	50.9	81.2	84.8	73.5
Blanc du Massif Central sheep	75.7	67.1	59.3	75.5	68.8	69.3
LSD$_{0.05}$	-	-	-	-	-	ns

In the next months the daily liveweight gain of this breed was lower. The lambs of the Wielkopolska sheep had lower liveweight gain compared to Romanov sheep, but the means for the grazing season between these breeds were not statistically significant. The lambs of Wielkopolska sheep had the highest liveweight gain in summer when the pasture yield was greatest. Lamb liveweight gain of the other two breeds, white-headed meat sheep and Blanc du Massif Central sheep, were significantly lower. In 2012 the best results for whole grazing season were for the Wielkopolska sheep. Slightly lower, but not significantly different, liveweight gain was observed for the white-headed meat sheep. Lambs of the Romanov sheep had lower liveweight gain, particularly in summer. In 2013 the liveweight gains of the four breeds did not differ significantly and were lower in comparison with previous years. It could be concluded the lambs of each breed showed in each month specific rhythm of pasture sward intake, which is dependent on the availability of fodder, its botanical and chemical composition and also on weather conditions. A good example is provided by the liveweight gains of lambs of all breeds in September: in 2011 this was the lowest in the whole season and in 2012 the highest. There are probably other conditions besides those evaluated in this study that influenced the differences in liveweight gain such occurrence of fungal diseases on the sward (Niżnikowski *et al.*, 2010).

Conclusion

Wielkopolska sheep and Romanov sheep performed well. Wielkopolska sheep had liveweight gains that were not statistically different from Romanov in 2011 and 2013, and the highest liveweight gain in 2012. Romanov had the highest liveweight gain in 2011. Similar to Wielkopolska sheep is the white-headed meat sheep. These two breeds are suitable for efficient lamb production on lowland pasture in Poland. As a native breed the Romanov sheep is better for utilization of low quality pasture and can play an important role in environmental protection and management of agricultural landscapes, whereas Wielkopolska sheep prefer a good yielding sward and continuous stocking with intensive regrowth during favourable weather conditions for efficient lamb production. Blanc du Massif Central sheep did not appear well adapted for grazing conditions in Poland.

Acknowledgements

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Appreciation of the functions of grassland by Belgian stakeholders

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Abstract

The European project MultiSward studied the appreciation of different functions of grasslands by European stakeholders. This paper describes the importance of grasslands for stakeholders in Belgium. Belgium currently has 578 504 ha of grassland, which is 43.3% of the total agriculturally utilized area. Belgian stakeholders appreciate grasslands especially for feed protein delivery at farm level, as a source of high quality forage and low cost animal feed especially for dairy milk production under grazing conditions. The most appreciated ecological aspects are conservation of ecosystems and biodiversity, erosion control and beauty of the landscape.

Keywords: grassland, stakeholders, Belgium

Introduction

Grasslands cover 43.3% (578 504 ha) of the agricultural area (AA) in Belgium: 38.0% is permanent grassland and 5.3% is temporary grassland. The area and the proportion of grasslands are higher in Wallonia (350 000 ha and 49% of AA) than in Flanders (228 400 ha and 37% of the AA) (Anonymous, 2014). The main forage production system in Flanders is based on regularly resown pastures and on annual forage crops (temporary pastures and silage maize). The system in Wallonia is based mainly on permanent pastures. Extensive grasslands are rare in Belgium. Intensive grasslands are dominated by *Lolium perenne* L. Compared with the European average, cattle intensification level is high to very high. The aim of the current study was to get an insight into the importance of grasslands for stakeholders in Belgium.

Materials and methods

An on-line questionnaire on functions of grasslands was distributed throughout Europe. This study provides results for Belgian stakeholders. A detailed description of the method can be found in Van den Pol-van Dasselaar *et al.* (2014, this volume). In Belgium, the questionnaire was distributed to members of the ILVO network, Landbouwcentrum voor Voedergewassen and the FP7-project Autograssmilk. In addition, it was spread via social media.

Results and discussion

When the questionnaire closed, 209 valid responses had been obtained. The majority of responses came from farmers (77 responses, or 37% of total response), followed by researchers (32 or 15%), advisers (30 or 14%), students (29 or 14%). policy makers (17 or 8%), industry (13 or 6%), education (6 or 3%) and NGOs (5 or 2%). The answers are grouped into four groups of ecosystem services: provisioning, regulating, supporting and cultural services. For the Belgian stakeholders the main provisioning service is to deliver high quality forage at a low cost for animal feed, mainly used for dairy milk production (Table 1).

Table 1. Importance of provisioning services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important)

	Advice	Edu-cation	Far-mers	Indus-try	NGO	Policy maker	Research	Stu-dents
High quality forage	4.2	4.0	4.4	4.1	4.0	3.9	4.3	4.0
Dairy cow milk production	4.2	3.7	4.3	4.2	4.6	4.1	4.5	4.0
Low cost animal feed	4.2	4.8	4.4	4.1	4.8	3.9	4.4	3.7
Nutritional quality of animal products for human consumption	3.8	4.5	3.8	3.5	3.4	3.9	4.1	3.7
Beef meat production	4.1	3.8	3.7	3.9	4.4	3.9	4.0	3.8
Global food production	3.7	3.7	3.6	3.5	3.2	3.1	3.7	3.8
Region of origin of animal products	3.8	3.7	2.9	3.0	3.0	3.0	3.5	3.6
Honey production	2.5	2.8	2.3	2.2	1.8	2.3	2.4	3.0
Sheep meat production	2.7	2.3	2.1	2.5	1.8	2.2	2.1	2.8
Biomass for energy production	2.1	2.3	1.7	2.0	1.8	2.1	1.9	2.5
Sheep milk production	2.2	2.2	2.0	2.2	2.0	2.0	1.8	2.4
Goat milk production	2.5	2.5	2.1	2.3	2.0	1.9	2.1	2.1
Wool production	2.1	1.7	1.7	1.8	1.6	1.9	1.7	2.3
Goat meat production	2.2	2.2	1.6	2.1	1.6	1.9	1.8	2.0
Production of plant fibre	1.8	2.0	1.5	1.9	1.4	1.6	1.6	2.6

Table 2. Importance of regulating services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important)

	Advice	Edu-cation	Far-mers	Indus-try	NGO	Policy maker	Research	Stu-dents
Biodiversity	3.7	3.8	2.9	3.2	3.8	3.5	3.8	3.8
Conservation of ecosystems quality	3.3	4.0	3.1	3.2	4.0	3.5	4.1	3.9
Water catchment	3.6	3.2	2.9	2.4	3.2	3.2	3.6	3.2
Erosion control	3.5	3.7	3.3	3.3	3.8	3.6	3.9	3.3
Carbon sequestration	3.7	3.5	3.3	3.3	3.0	3.9	4.1	3.4
Mitigating greenhouse gas emissions	3.5	3.5	2.7	3.2	2.4	3.1	3.5	3.2
Adaptation to climate change	3.1	3.5	2.7	2.6	2.0	3.0	3.4	3.1
Flood plains rivers	2.9	2.3	2.4	2.4	2.8	3.1	3.2	3.1
Pathogen control in cropping system	3.4	3.5	3.2	3.6	2.2	3.6	3.6	3.5
Fire control	2.1	1.8	2.0	2.2	1.2	2.1	1.8	2.8
Avalanche control	1.8	1.5	1.5	1.2	2.4	1.6	1.5	1.8

Table 3. Importance of cultural services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important)

	Advice	Edu-cation	Far-mers	Indus-try	NGO	Policy maker	Research	Stu-dents
Beauty of the landscape	3.5	4.0	3.3	3.4	4.2	3.3	4.0	3.7
Positive perception of animal production systems	4.1	4.7	3.8	3.8	4.6	3.4	4.1	3.7
Rural development	3.4	3.2	2.7	2.8	2.6	3.1	3.6	3.0
Maintaining population in rural areas	3.4	4.0	3.1	3.4	3.2	3.2	3.8	3.5
Cultural values	2.7	4.0	2.2	2.5	3.2	2.8	3.1	2.7
Tourism / recreation	3.0	3.2	2.1	2.4	3.8	2.8	3.0	2.4
Supporting horses for equestrian sport and recreation	2.2	2.2	1.6	2.0	1.6	2.5	2.4	3.1

The most important regulating services are conservation of ecosystems, biodiversity and erosion control (Table 2). Belgian stakeholders establish the important role of grassland in the positive perception of animal production systems and beauty of the landscape. The score for tourism/recreation is rather low (Table 3). The most important supporting services are grazing and feed protein supply at farm level (Table 4).

Table 4. Importance of supporting services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important)

	Advice	Education	Farmers	Industry	NGO	Policy maker	Research	Students
Grazing	4.3	4.5	4.2	3.6	4.0	4.0	4.5	4.3
Animal health	3.9	4.2	4.2	3.9	3.4	3.3	3.8	4.4
Animal welfare	3.7	3.8	4.0	3.9	3.4	3.2	3.8	4.3
Conservation of soil structure and fertility in cropping systems	4.1	3.2	3.8	3.5	4.0	4.2	4.0	3.9
Feed protein supply at farm level	4.4	4.4	4.2	3.5	4.0	3.9	4.5	3.4
Competitiveness of farming systems	3.6	3.7	3.3	3.2	3.8	2.9	3.6	2.9
N fixation via legumes	3.7	4.0	3.4	3.0	3.4	3.5	4.0	3.4
Availability of water	3.4	3.0	3.1	2.9	2.8	3.1	3.4	3.7
Crop pollination	2.9	3.0	2.5	2.5	2.2	2.6	2.9	3.6

Conclusion

Belgian stakeholders appreciate grassland especially for feed protein delivery at farm level, as a source of high quality forage and low cost animal feed especially for dairy milk production under grazing conditions. The most appreciated characteristics are conservation of ecosystems and biodiversity, erosion control and beauty of the landscape. Grasslands induce a positive perception of animal production systems and are considered as essential components of animal health and welfare.

Acknowledgements

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Appreciation of the functions of grassland by Dutch stakeholders

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Abstract

The European project MultiSward studied the appreciation of different functions of grasslands by European stakeholders. This paper describes the importance of grasslands for stakeholders in the Netherlands. There is currently 1 million ha of grassland in the Netherlands, which is 40-45% of the total agriculturally utilized area. Dutch stakeholders appreciate the different functions of grasslands, especially high quality forage, dairy cow milk production, low cost animal feed and grazing. Functions that are less relevant for the Netherlands, like sheep and goat production, fire control and avalanche control, are also less appreciated. We conclude that stakeholders appreciate grasslands in the Netherlands as a valuable resource for many ecosystem services.

Keywords: grassland, stakeholders, the Netherlands

Introduction

Grasslands cover almost 1 million ha in the Netherlands, which is 40-45% of the total agricultural area. The majority of these grasslands (75%) are permanent grasslands; 20% consists of temporary grasslands and 5% is natural grasslands (CBS, 2014). Grasslands are usually intensively managed and dominated by perennial ryegrass (*Lolium perenne* L.). The grasslands are used as feed for dairy cattle. The Dutch dairy sector is characterized by relatively high levels of supplementation, mainly silage maize and concentrates. Silage maize covers about 10% of the total agricultural area in the Netherlands (CBS, 2014) and is fully used for dairy feed. About 70% of the dairy cattle are grazing for at least part of the grazing season (CBS, 2014). The aim of the current study was to obtain an insight into the importance of grasslands for stakeholders in the Netherlands.

Materials and methods

An on-line questionnaire on the appreciation of functions of grasslands was distributed throughout Europe. A detailed description of the method can be found in Van den Pol-van Dasselaar *et al.* (2014, this volume). This paper describes the results for the Netherlands. In the Netherlands, the questionnaire was distributed to members of the Netherlands Society for Grassland and Fodder Crops and the Dutch farmers' association LTO. In addition, it was distributed via social media, like LinkedIn.

Results and discussion

At the time of closing the questionnaire, 206 valid responses had been obtained. The majority of these responses came from farmers (90 responses, which equals 44% of total response), followed by advisers (34; 17%), researchers (30; 15%), industry (29; 14%), policy makers (8; 4%), education (8; 4%) and NGOs (5; 2%). Students were not included, since only 2 students responded. Tables 1 to 4 show the appreciation of the functions of grasslands by the different stakeholders. The functions are grouped into the four groups of ecosystem services: provisioning services, regulating services, supporting services and cultural services. Many provisioning services are highly appreciated by Dutch stakeholders but a number of them, such as sheep and goat production, are less relevant under Dutch conditions. This is also true for some regulating functions like fire control and avalanche control. Cultural services, such as the contribution of grasslands to the beauty of the landscape and the perception of animal production systems, are seen as important. With respect to supporting services, animal health

is valued highly, especially by farmers. Farmers and policy makers consider feed protein supply at farm-level also to be an important function of grasslands.

Table 1. Importance of provisioning services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Policy maker	Research
High quality forage	4.4	4.0	4.8	4.6	4.0	4.0	4.5
Dairy cow milk production	4.6	4.3	4.5	4.6	4.2	4.6	4.7
Low cost animal feed	4.4	3.8	4.6	4.3	3.2	3.9	4.1
Nutritional quality of animal products for human consumption	3.9	3.6	3.9	4.0	3.2	3.8	3.9
Beef meat production	2.9	2.8	3.0	3.7	2.8	2.4	3.3
Global food production	3.6	3.8	4.0	4.2	2.6	3.5	3.7
Region of origin of animal products	3.5	3.4	2.9	3.6	3.6	3.5	3.1
Honey production	2.4	2.8	2.0	2.3	2.8	2.6	2.1
Sheep meat production	2.1	2.6	2.1	2.9	3.2	1.9	2.3
Biomass for energy production	1.8	2.3	1.5	2.3	2.0	1.8	2.1
Sheep milk production	2.1	2.4	1.7	2.6	3.2	1.5	2.0
Goat milk production	2.3	2.1	1.8	2.8	2.8	1.8	2.0
Wool production	1.8	2.0	1.7	2.2	2.8	2.1	2.0
Goat meat production	1.8	1.6	1.4	2.3	2.6	1.3	1.7
Production of plant fibre	1.8	1.8	1.5	2.2	1.8	2.3	1.9

Table 2. Importance of regulating services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Policy maker	Research
Biodiversity	3.6	4.1	3.2	3.3	4.4	3.5	3.7
Conservation of ecosystems quality	3.8	4.0	3.3	3.4	4.6	3.9	3.4
Water catchment	3.3	2.8	2.4	3.2	2.8	3.3	3.2
Erosion control	2.6	2.9	2.3	3.0	3.5	2.4	2.5
Carbon sequestration	3.3	2.8	2.9	3.2	4.0	3.9	3.5
Mitigating greenhouse gas emissions	3.3	3.8	2.9	2.9	4.0	3.9	3.2
Adaptation to climate change	3.5	2.7	2.8	2.8	3.4	3.6	3.2
Flood plains rivers	3.0	3.6	2.3	3.3	3.4	4.6	3.2
Pathogen control in cropping system	3.5	3.3	3.1	3.4	3.2	3.3	3.2
Fire control	1.9	1.8	1.7	2.2	2.4	1.6	1.8
Avalanche control	1.4	1.8	1.3	1.6	2.0	1.1	1.7

Table 3. Importance of cultural services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Edu-cation	Far-mers	Indus-try	NGO	Policy maker	Research
Beauty of the landscape	3.9	4.1	3.8	3.6	4.2	4.0	4.1
Positive perception of animal production systems	4.4	3.8	4.1	3.9	4.2	4.3	3.9
Rural development	3.3	3.1	3.3	3.3	3.6	3.0	3.2
Maintaining population in rural areas	3.5	3.8	3.7	3.6	4.2	3.6	3.5
Cultural values	3.6	3.3	2.9	3.2	3.4	4.0	3.6
Tourism / recreation	3.1	3.9	2.7	3.1	3.2	3.3	3.4
Supporting horses for equestrian sport and recreation	2.1	2.1	1.7	2.8	3.3	2.0	2.3

Table 4. Importance of supporting services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Edu-cation	Far-mers	Indus-try	NGO	Policy maker	Research
Grazing	4.5	3.8	4.2	4.3	4.0	4.8	4.3
Animal health	4.0	3.9	4.4	4.1	3.8	3.9	4.0
Animal welfare	3.9	3.9	4.0	4.0	4.2	4.1	3.7
Conservation of soil structure and fertility in cropping systems	4.1	4.1	4.0	3.9	3.6	4.0	4.0
Feed protein supply at farm level	4.4	3.9	4.7	4.3	3.8	4.8	4.4
Competitiveness of farming systems	3.5	3.3	3.7	3.6	2.8	3.9	3.9
N fixation via legumes	3.4	4.1	3.2	3.4	3.4	3.9	3.3
Availability of water	3.4	3.8	3.5	3.6	3.8	3.4	3.3
Crop pollination	2.6	2.7	2.4	2.6	3.6	2.8	2.3

Conclusion

Dutch stakeholders appreciate the different functions of grasslands, especially high quality forage, dairy cow milk production and grazing. Functions that are less relevant for the Netherlands are also less appreciated. We conclude that stakeholders appreciate grasslands in the Netherlands as a valuable resource for many ecosystem services.

Acknowledgements

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Appreciation of the functions of grassland by French stakeholders

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Abstract

The European project MultiSward studied the appreciation of different functions of grasslands by European stakeholders. This paper describes the importance of grasslands for stakeholders in France. France currently has 11 million ha grasslands, which is 38% of the total agriculturally utilized area. French stakeholders especially appreciate the functions of production of forage quality, low production costs and production of protein as well as the suitability for grazing or biodiversity production. Functions like production of biomass for energy production or for fibre are less appreciated. We conclude that French stakeholders recognize the high potential of grasslands for developing animal production systems that are economically viable and environment-friendly.

Keywords: grassland, stakeholders, France

Introduction

France has a very large acreage devoted to permanent and temporary grasslands, contributing to a total of 11 million ha (38% of the total agricultural utilized area). Annual forage crops are very important, especially silage maize (5.5% of the total agricultural utilized area). At present, most temporary grasslands are sown with mixtures of grasses and legumes producing large amounts of protein-rich feed. They also include a significant acreage of lucerne in some specific regions, grown as pure crops, although this acreage has declined since 1990. Permanent grasslands are abundant, especially in mountainous areas and hilly regions, where they are the main feed resources for the large herds of suckler-cows, but also in Normandy and eastern France for dairy herds where it is not possible to plough. Despite an important reduction in the last three decades, grasslands are a major component of most French landscapes. Dairy farming is important in lowland areas of the western part of France and also in the mountainous regions in the East (Franche-Comté and Alps) and in the Massif Central. In these regions, milk is processed to produce PDO cheese. High milk yield and animal performances and low production costs are key issues for the farmers, who are concerned by the work load and by the preservation of the environment. The aim of the current study was to get an insight into the appreciation of the functions of grasslands by stakeholders in France.

Materials and methods

This study provides results that were obtained via an on-line questionnaire on the functions of grasslands and which was distributed throughout Europe. A detailed description of the method can be found in Van den Pol-van Dasselaar *et al*. (2014, this volume). This paper describes results for French stakeholders. In France, the questionnaire was distributed to members of the French Grassland Society (AFPF) and promoted during conferences of this society.

Results and discussion

At the time of closing the questionnaire, 356 valid responses had been obtained from France. The majority of these responses came from advisers (142 responses, which equals 40% of total response) followed by research (116; 32%), industry (24; 7%), farmers (24; 7%), policy makers (21; 6%), education (17; 5%) and students (10; 3%). Three responses only were provided by

members of NGOs and their ratings will be little discussed. Tables 1 to 4 show the appreciation of the functions of grasslands by the different stakeholders. French stakeholders gave a well-balanced appreciation across the various ecosystem services: provisioning services, regulating services, supporting services and cultural services.

Table 1. Importance of provisioning services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Policy maker	Research	Students
High quality forage	4.1	3.8	4.1	3.6	4.3	4.1	3.7	4.2
Dairy cow milk production	4.0	3.6	3.8	4.0	3.0	4.0	3.8	4.2
Low cost animal feed	4.3	3.9	4.1	3.7	2.7	4.4	4.0	4.4
Nutritional quality of animal products for human consumption	3.7	3.5	4.0	3.2	4.3	4.4	3.7	3.9
Beef meat production	4.2	3.8	4.0	4.0	3.7	4.3	3.7	3.6
Global food production	3.8	3.4	3.6	3.3	3.7	3.8	3.5	3.3
Region of origin of animal products	3.5	3.6	3.7	3.5	4.0	3.8	3.5	3.0
Honey production	2.8	2.9	2.5	2.7	3.0	2.9	2.9	2.7
Sheep meat production	3.4	2.8	3.1	3.4	2.7	3.7	3.1	2.4
Biomass for energy production	1.9	2.1	1.7	2.3	2.3	1.8	2.0	2.2
Sheep milk production	2.4	2.9	2.1	2.9	2.0	2.2	2.4	2.2
Goat milk production	2.7	2.7	2.4	2.5	2.3	2.7	2.4	2.1
Wool production	1.8	2.0	1.8	2.2	2.0	1.8	1.7	2.0
Goat meat production	1.7	2.0	1.8	2.3	1.3	1.9	1.8	2.1
Production of plant fibre	1.6	1.6	1.7	2.1	1.7	1.6	1.8	1.4

Table 2. Importance of regulating services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Policy maker	Research	Students
Biodiversity	4.1	4.3	3.9	3.6	4.7	4.6	4.2	4.3
Conservation of ecosystems quality	3.9	3.9	3.6	3.7	4.0	4.5	4.0	3.7
Water catchment	4.0	4.1	3.7	3.9	4.0	3.9	3.9	3.2
Erosion control	3.6	3.5	3.7	3.7	2.7	3.8	3.6	3.8
Carbon sequestration	3.5	3.6	3.5	3.3	2.0	3.8	3.8	3.2
Mitigating greenhouse gas emissions	3.5	3.2	3.3	3.3	2.3	3.5	3.4	3.1
Adaptation to climate change	3.4	3.6	3.8	3.3	3.0	3.7	3.3	3.0
Flood plains rivers	2.9	3.3	3.4	3.0	3.3	3.5	3.0	2.3
Pathogen control in cropping system	3.1	3.0	3.1	3.0	2.0	3.0	3.0	3.4
Fire control	2.2	2.0	2.4	2.4	3.0	2.5	2.2	2.4
Avalanche control	1.8	1.7	1.6	1.9	2.3	2.0	2.1	1.6

Table 3. Importance of cultural services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Policy maker	Research	Students
Beauty of the landscape	4.0	3.9	4.2	3.2	4.7	4.3	3.9	3.8
Positive perception of animal production systems	4.2	3.9	4.0	3.8	4.0	4.4	4.0	3.4
Rural development	3.3	3.6	3.2	3.2	3.7	3.9	3.4	3.6
Maintaining population in rural areas	3.2	3.7	3.5	3.0	3.7	4.1	3.4	3.3
Cultural values	3.4	3.2	3.0	2.8	4.3	3.9	3.4	2.8
Tourism / recreation	3.2	3.6	3.2	2.8	4.0	3.8	3.3	2.5
Supporting horses for equestrian sport and recreation	2.2	2.1	2.1	2.3	3.0	2.4	2.3	2.1

Table 4. Importance of supporting services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Policy maker	Research	Students
Grazing	4.6	4.3	4.3	4.0	3.7	4.5	4.4	4.6
Animal health	3.7	3.4	4.0	3.3	3.7	3.6	3.4	3.5
Animal welfare	3.9	3.4	4.1	3.2	3.7	3.8	3.5	4.1
Conservation of soil structure and fertility in cropping systems	3.9	3.6	4.0	3.9	4.3	3.9	3.9	4.1
Feed protein supply at farm level	4.0	3.9	4.3	3.6	3.0	4.0	3.7	3.8
Competitiveness of farming systems	3.9	3.6	4.0	3.5	2.3	4.0	3.6	3.3
N fixation via legumes	4.0	3.9	4.0	3.8	2.3	3.7	3.7	4.3
Availability of water	3.2	2.8	3.4	3.3	2.7	3.4	3.3	3.1
Crop pollination	2.9	3.2	3.0	3.2	2.0	3.0	3.2	3.1

Conclusion

French stakeholders provided a high number of responses to the on-line questionnaire. There were very subtle differences among groups of stakeholders, and this means that their views related to the most important functions are commonly shared and that a policy in favour of grasslands would probably be approved by all stakeholder groups. Provisioning and supporting services are considered as essential. But regulating services, especially biodiversity, and cultural services are also identified as important.

Acknowledgements

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Appreciation of the functions of grassland by Irish stakeholders

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Abstract

The European project MultiSward studied the appreciation of different functions of grasslands by European stakeholders. This paper describes the importance of grasslands for stakeholders in Ireland. Ireland currently has approximately 4.6 million ha of grassland, which is 90% of the total utilized agricultural area. Irish stakeholders consider grassland to be important for a range of functions and services including milk and meat production, forage production, animal health and welfare, perception of animal production systems and biodiversity. Functions like goat meat production, production of plant fibre, fire control and avalanche control are less appreciated. All stakeholder groups generally agreed on the importance of the functions evaluated.

Keywords: grassland, stakeholders, Ireland

Introduction

Ireland has a grassland area of approximately 4.6 million ha of grassland (consisting of pasture, grass silage or hay, and rough grazing), which is 90% of the total utilizable agricultural area (O'Mara, 2008). Ruminant production systems (milk and meat) in Ireland are predominantly grass based and Ireland has a long grazing season (February/March to October/November, depending on soil type and rainfall). Grazed grass is the main feed source during the grazing season, and winter forage consists predominantly of grass silage. Grassland in Ireland is predominately permanent pasture and the proportion of agricultural land reseeded (from grass to grass) annually is low at approximately 2% (Shalloo *et al.*, 2011). Perennial ryegrass is the most widely sown grass species. The aim of the current study was to get an insight into the importance of grasslands for stakeholders in Ireland.

Materials and methods

This study provides results for Irish stakeholders obtained via an on-line questionnaire on functions of grasslands. The questionnaire was distributed throughout Europe. A detailed description of the method can be found in Van den Pol-van Dasselaar *et al.* (2014, this volume). This paper describes the results for Ireland. In Ireland the questionnaire was distributed to members of the Irish Grassland Association, Teagasc (researchers, students, technicians, advisers), Universities, members of farming organizations, members of government departments and farmers.

Results and discussion

Two hundred and thirty-two valid responses were obtained. The majority of these responses came from advisers (67 responses; 29% of total), followed by researchers (66; 28%), farmers (37; 16%), students (26; 11%), education (23; 10%), policy makers (8; 3%), industry (3; 1%) and NGOs (2; <1%). Tables 1 to 4 show the appreciation of the functions of grasslands by the different stakeholders. The functions are grouped into the four groups of ecosystem services: provisioning services, regulating services, supporting services and cultural services. The provisioning services (Table 1) that Irish stakeholders consider to be most important include milk and meat (beef and sheep) production, the production of high quality forage, and nutritional quality of animal products for human consumption. Biodiversity and conservation

of quality of ecosystems are considered to be important regulating services (Table 2), and NGOs and policy makers consider these to be more important than do the other stakeholder categories. Fire and avalanche control are not very important in Ireland. Positive perception of animal production systems, beauty of the landscape, and maintaining population in rural areas are the most important cultural services of grassland (Table 3). Grazing, competitiveness of farming systems, and animal health and welfare are considered the most important supporting services (Table 4). In general, across the functions evaluated, there was good agreement between all stakeholder groups.

Table 1. Importance of provisioning services of grasslands according to the respondents (by stakeholder category) of the questionnaire (1 = not important; 5 = very important).

	Advice	Edu-cation	Far-mers	Indus-try	NGO	Policy maker	Research	Stu-dents
High quality forage	4.6	4.5	4.6	4.7	4.5	4.1	4.5	4.6
Dairy cow milk production	4.7	4.5	4.5	4.3	5.0	4.4	4.5	4.3
Low cost animal feed	4.6	4.4	4.7	5.0	4.5	4.4	4.5	4.5
Nutritional quality of animal products for human consumption	4.3	4.2	4.6	4.7	5.0	4.0	4.2	4.3
Beef meat production	4.6	4.2	4.1	4.3	4.5	4.4	4.5	4.5
Global food production	4.2	4.0	4.1	4.0	4.5	4.1	4.1	4.2
Region of origin of animal products	3.5	3.5	3.8	4.3	3.5	3.4	3.3	3.7
Honey production	1.8	2.6	2.0	1.0	3.5	2.1	2.1	2.1
Sheep meat production	3.6	3.2	2.9	2.0	4.0	3.9	3.3	3.5
Biomass for energy production	1.8	2.2	2.0	1.7	2.5	1.6	2.2	2.7
Sheep milk production	1.8	1.7	1.7	2.0	3.5	2.1	1.8	2.1
Goat milk production	1.6	1.5	1.5	1.0	3.5	1.5	1.5	1.8
Wool production	2.3	2.3	2.1	1.3	3.5	2.4	2.0	2.5
Goat meat production	1.5	1.7	1.5	1.0	1.5	1.3	1.3	1.8
Production of plant fibre	1.6	1.8	1.7	1.0	3.5	1.4	1.6	2.3

Table 2. Importance of regulating services of grasslands according to the respondents (by stakeholder category) of the questionnaire (1 = not important; 5 = very important).

	Advice	Edu-cation	Far-mers	Indus-try	NGO	Policy maker	Research	Stu-dents
Biodiversity	3.4	3.9	3.0	2.7	5.0	4.5	3.3	3.4
Conservation of ecosystems quality	3.2	3.9	3.2	3.0	4.5	4.4	3.5	3.4
Water catchment	3.4	3.8	3.3	2.0	4.0	4.1	3.2	3.4
Erosion control	2.3	2.9	2.8	1.3	2.0	3.3	2.8	2.7
Carbon sequestration	3.0	3.1	2.8	2.3	4.0	3.9	3.3	3.3
Mitigating greenhouse gas emissions	3.3	3.1	2.7	2.3	4.0	4.1	3.2	3.2
Adaptation to climate change	3.1	3.2	3.4	3.0	3.0	3.6	3.1	3.5
Flood plains rivers	2.6	3.1	2.9	1.3	3.5	3.5	2.8	2.9
Pathogen control in cropping system	2.4	2.7	3.1	2.3	4.0	3.0	2.5	2.8
Fire control	1.7	1.9	2.5	1.0	1.5	1.6	1.7	2.2
Avalanche control	1.1	1.5	1.6	1.0	1.0	1.0	1.2	2.0

Table 3. Importance of cultural services of grasslands according to the respondents (by stakeholder category) of the questionnaire (1 = not important; 5 = very important).

	Advice	Edu-cation	Far-mers	Indus-try	NGO	Policy maker	Research	Stu-dents
Beauty of the landscape	3.7	4.0	3.8	3.3	4.5	4.3	3.7	3.6
Positive perception of animal production systems	4.4	4.1	4.2	4.0	4.0	4.4	4.1	3.9
Rural development	3.1	3.3	3.8	3.7	3.5	4.0	3.4	3.7
Maintaining population in rural areas	3.7	3.9	4.0	3.7	2.5	4.1	3.5	3.8
Cultural values	2.6	3.7	3.1	3.0	4.0	3.6	3.0	3.2
Tourism / recreation	2.8	3.5	2.7	2.7	3.0	4.0	3.4	3.1
Supporting horses for equestrian sport and recreation	2.4	2.5	2.4	3.3	3.5	3.4	2.8	2.5

Table 4. Importance of supporting services of grasslands according to the respondents (by stakeholder category) of the questionnaire (1 = not important; 5 = very important).

	Advice	Edu-cation	Far-mers	Indus-try	NGO	Policy maker	Research	Stu-dents
Grazing	4.9	4.5	4.7	5.0	5.0	4.8	4.6	4.7
Animal health	4.3	4.3	4.6	5.0	4.5	4.0	4.0	4.1
Animal welfare	4.1	4.1	4.5	5.0	3.5	4.1	3.9	4.0
Conservation of soil structure and fertility in cropping systems	3.8	3.6	3.9	4.0	4.5	4.0	3.4	4.0
Feed protein supply at farm level	3.8	3.7	4.2	4.0	4.5	3.5	3.7	4.2
Competitiveness of farming systems	4.6	3.6	4.4	5.0	5.0	4.5	4.2	4.2
N fixation via legumes	2.9	3.4	3.3	2.3	2.0	3.5	3.2	3.6
Availability of water	2.8	3.3	3.5	1.7	4.0	3.1	3.0	3.8
Crop pollination	2.5	3.0	3.2	2.7	4.0	3.3	2.9	3.2

Conclusion

A wide range of stakeholders responded to the survey in Ireland. Stakeholders consider grassland to be important for a range of functions and services including bovine milk production, beef and sheep meat production, forage production, animal health and welfare, perception of animal production systems and biodiversity. The least important functions considered by the stakeholders include goat meat production, production of plant fibre, fire control and avalanche control. All stakeholder groups generally agreed on the importance of the functions evaluated.

Acknowledgements

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Appreciation of the functions of grassland by Italian stakeholders

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Abstract

The European project MultiSward studied the appreciation of different functions of grasslands by European stakeholders. This paper describes the importance of grasslands for stakeholders in Italy. Italy currently has approximately 6.0 million ha of grassland, consisting of permanent grassland, pastures and temporary grassland, which is 47% of the total agriculturally utilized area (12.9 million ha). Italian stakeholders considered grassland to be important for a range of functions and services but they especially appreciate conservation of the quality of ecosystems and biodiversity such as beauty of the landscape and cultural values. Important functions like carbon sequestration and mitigating greenhouse gas emissions are less known and appreciated. All stakeholder groups generally agreed on the importance of the functions evaluated.

Keywords: grassland, stakeholders, Italy

Introduction

Italy has a grassland area of approximately 6.0 million ha of grassland (consisting of permanent grassland, pasture and temporary grassland), which is 47% of the total utilizable agricultural area (12.9 million ha). Ruminant production systems are predominantly grass-based in the mountain areas and in southern Italy, and based on maize silage on the plains of northern Italy (e.g. Po valley). Cattle (5.7 million), sheep (6.6 million) and goats (about 0.8 million) are the most important types of animals reared. The most important grassland species cultivated are sainfoin in southern Italy and lucerne in central and northern Italy (INEA, 2012; ISTAT, 2010). The aim of the current study was to obtain an insight into the appreciation of the functions of grasslands by stakeholders in Italy.

Materials and methods

This study provides results that were obtained via an on-line questionnaire on the functions of grasslands and which was spread throughout Europe. A detailed description of the method can be found in Van den Pol-van Dasselaar *et al*. (2014, this volume). This paper describes the results for Italy. In Italy, the questionnaire was distributed and collected during national and international meetings held in different regions of Italy, to the Alpine Zootechnical Society (SooZooAlp), University of Udine (researchers, students, technicians, advisers), Universities, members of farming organizations, members of government departments and farmers. In addition, it was spread via social media. The majority of the answers to the questionnaires have been collected during the meetings and sent to the elaboration centre.

Results and discussion

At the time of closing the questionnaire, 241 valid responses were obtained from Italy. The majority of the 241 valid responses came from students (117 responses, which equals 49% of total responses), followed by NGO (30; 12%), education (24; 10%), policy makers (22; 9%), researchers (20; 8%), advice (14; 6%), farmers (11; 5%), and industry (3; 1%). Tables 1 to 4 show the appreciation of the functions of grasslands by the different stakeholders. The functions are grouped into the four groups of ecosystem services: provisioning services, regulating services, supporting services and cultural services.

Table 1. Importance of provisioning services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Policy maker	Research	Students
High quality forage	4.1	4.0	3.7	3.7	3.8	4.3	3.8	3.5
Dairy cow milk production	3.3	3.4	3.3	2.7	3.5	3.4	3.5	3.6
Low cost animal feed	3.6	3.1	2.9	2.0	2.7	4.1	3.5	3.0
Nutritional quality of animal products for human consumption	3.6	3.9	3.5	2.7	3.7	4.3	3.5	3.7
Beef meat production	2.8	2.8	3.6	3.7	3.0	3.2	3.0	3.7
Global food production	2.7	3.1	3.4	3.0	2.8	3.3	2.8	3.4
Region of origin of animal products	3.5	3.7	3.5	3.0	3.5	3.9	3.8	3.8
Honey production	3.5	3.6	3.2	3.7	3.8	3.7	3.4	3.5
Sheep meat production	2.1	2.5	2.8	2.7	2.3	3.1	2.9	3.0
Biomass for energy production	2.4	2.2	2.7	3.0	2.1	2.1	2.0	3.2
Sheep milk production	1.9	2.5	2.3	2.7	2.4	3.7	2.9	2.8
Goat milk production	2.3	2.1	1.9	2.7	2.6	3.1	2.7	2.6
Wool production	1.5	2.0	1.8	2.0	2.0	2.2	2.2	2.5
Goat meat production	2.4	2.0	2.1	2.7	2.3	2.5	2.6	2.7
Production of plant fibre	2.4	2.1	2.2	2.0	1.8	1.7	2.0	2.9

Table 2. Importance of regulating services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Policy maker	Research	Students
Biodiversity	4.6	4.7	3.7	3.0	4.5	4.7	4.1	3.9
Conservation of ecosystems quality	4.7	4.7	4.1	3.0	4.5	4.4	4.0	4.0
Water catchment	4.3	3.7	3.3	3.3	3.5	3.6	3.3	3.4
Erosion control	4.1	3.9	3.3	4.3	3.7	4.2	3.6	3.3
Carbon sequestration	3.6	3.6	2.9	3.0	3.3	3.3	3.2	3.0
Mitigating greenhouse gas emissions	3.2	3.5	3.1	2.3	3.1	3.5	3.0	3.2
Adaptation to climate change	3.4	3.8	3.3	2.3	3.4	3.3	2.7	3.4
Flood plains rivers	3.9	3.7	3.5	3.3	3.2	3.0	2.9	3.5
Pathogen control in cropping system	3.3	3.1	2.7	2.7	3.2	3.1	2.5	3.4
Fire control	3.4	3.7	3.4	3.3	3.2	3.4	3.1	3.4
Avalanche control	3.4	3.0	2.7	2.0	2.9	3.0	2.2	3.2

Table 3. Importance of cultural services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Edu-cation	Far-mers	Indus-try	NGO	Policy maker	Research	Stu-dents
Beauty of the landscape	4.6	4.8	4.3	4.0	4.4	4.5	4.0	4.0
Positive perception of animal production systems	3.7	3.4	2.8	3.3	3.4	4.0	3.7	3.4
Rural development	3.9	3.6	4.0	3.0	3.6	3.9	3.4	3.4
Maintaining population in rural areas	3.9	3.4	4.1	2.7	3.4	4.2	3.2	3.3
Cultural values	4.0	4.3	4.3	2.7	4.0	4.4	3.7	3.7
Tourism / recreation	3.8	4.0	3.6	3.3	4.0	4.0	3.4	3.6
Supporting horses for equestrian sport and recreation	1.8	1.9	1.8	2.5	2.1	2.6	1.9	2.3

Table 4. Importance of supporting services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Edu-cation	Far-mers	Indus-try	NGO	Policy maker	Research	Stu-dents
Grazing	3.6	3.9	3.2	3.7	3.7	4.6	4.2	3.7
Animal health	3.9	4.2	4.0	3.0	3.8	3.8	3.5	3.8
Animal welfare	3.6	4.1	3.7	3.3	4.0	3.8	3.7	3.7
Conservation of soil structure and fertility in cropping systems	4.0	3.9	3.7	3.7	3.9	4.1	3.6	3.6
Feed protein supply at farm level	2.8	2.6	2.7	2.0	2.4	3.5	2.6	2.9
Competitiveness of farming systems	3.8	2.9	3.3	2.7	3.2	3.7	2.9	3.2
N fixation via legumes	3.3	3.5	3.1	3.0	2.9	3.5	3.0	3.2
Availability of water	3.4	3.6	4.0	3.0	3.3	4.0	2.9	3.7
Crop pollination	3.6	4.0	3.3	2.7	3.3	3.5	2.9	3.6

Conclusion

A wide range of stakeholders responded to the survey in Italy. Italian stakeholders considered grasslands to be important for a range of functions and services but especially appreciate conservation of the quality of ecosystems and biodiversity such as beauty of the landscape and cultural values. Important functions like carbon sequestration and mitigating greenhouse gas emissions are less known and appreciated. All stakeholder groups generally agreed on the importance of the functions evaluated.

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Appreciation of the functions of grassland by Polish stakeholders

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Abstract

The European project MultiSward studied the appreciation of different functions of grasslands by European stakeholders. This paper describes the importance of grasslands for stakeholders in Poland. Poland currently has 3.29 million ha of grassland, which is 21.3% of the total agriculturally utilized area. Polish stakeholders consider grassland to be important for a range of functions and services including animal health, dairy cow milk production, nutritional quality of animal products for human consumption, low cost animal feed, beauty of the landscape and biodiversity. Functions like goat milk and meat production, wool production, production of plant fibre and avalanche control are less appreciated.

Keywords: grassland, stakeholders, Poland

Introduction

Grasslands in Poland occupy a total area of 3.29 million hectares, which constitutes 21.3% of the total UAA or 12.6% of the entire area of the country. However, this area does not include leys established on arable land (temporary grasslands) covering 0.45 million ha, whose duration of utilization does not exceed 4-5 years (Goliński, 2014). Meadows represent 77% and pastures about 23% of the grassland area. The share of permanent pasture in Poland has decreased during the last 20 years by more than 50%. The number of cattle and sheep has also decreased. Several regional programmes have been initiated to stimulate economic development and preservation of cultural heritage. 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 (Warda and Kozłowski, 2012). The aim of the current study was to obtain an insight into the importance of grasslands for stakeholders in Poland.

Materials and methods

This study provides results for Polish stakeholders, which were obtained via an on-line questionnaire on the functions of grasslands that was spread throughout Europe. A detailed description of the method can be found in Van den Pol-van Dasselaar *et al*. (2014, this volume). In Poland, the questionnaire was distributed to members of the Polish Grassland Society (researchers, students, technicians, advisors), universities and institutions related to grassland, farming organizations, government departments, NGOs and farmers.

Results and discussion

At the time of closing the questionnaire, 204 valid responses had been obtained. The majority of these responses came from students (110 responses, which equals 54%), followed by researchers (42; 21%), farmers (20; 10%), advisers (15; 7%), education (9; 4%), industry (5; 2%) and NGOs (3; 2%). Policy makers were also included but no responses were received. Tables 1 to 4 show the appreciation of the functions of grasslands by the different stakeholders. The functions are grouped into the four groups of ecosystem services: provisioning services, regulating services, supporting services and cultural services. The provisioning services that Polish stakeholders consider to be most important include dairy cow milk production,

nutritional quality of animal products for human consumption and low cost animal feed. Biodiversity and conservation of the quality of ecosystems are considered to be important regulating services. Beauty of the landscape is the most important cultural service of grassland. Animal health and welfare are considered the most important supporting services. In general, across the functions evaluated, there was good agreement between all stakeholder groups.

Table 1. Importance of provisioning services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Research	Students
High quality forage	4.1	3.9	4.2	4.4	3.0	4.0	3.3
Dairy cow milk production	4.5	3.9	4.2	4.2	4.0	3.8	3.5
Low cost animal feed	4.3	4.0	4.2	3.8	3.7	4.0	3.4
Nutritional quality of animal products for human consumption	4.1	4.0	3.6	4.6	4.0	3.9	3.8
Beef meat production	3.5	3.3	3.9	2.8	2.7	3.2	2.9
Global food production	3.4	3.8	3.2	3.4	2.7	3.3	2.7
Region of origin of animal products	2.8	3.9	2.6	2.8	1.7	3.5	3.1
Honey production	3.1	2.8	3.4	2.6	2.7	3.0	3.1
Sheep meat production	2.0	2.3	1.4	1.6	2.0	2.2	2.0
Biomass for energy production	2.5	3.2	2.3	2.6	3.0	2.2	2.7
Sheep milk production	2.0	2.3	1.5	1.2	2.0	2.3	2.1
Goat milk production	1.8	2.4	1.6	1.2	2.0	1.9	1.9
Wool production	1.7	2.4	1.7	1.0	2.0	1.8	2.1
Goat meat production	1.8	1.9	1.5	1.4	2.0	1.7	1.9
Production of plant fibre	1.7	2.3	1.6	1.4	1.3	1.6	2.0

Table 2. Importance of regulating services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Research	Students
Biodiversity	3.6	4.0	4.2	3.4	3.7	4.4	3.9
Conservation of ecosystems quality	3.6	4.0	3.8	3.4	3.3	3.9	3.5
Water catchment	3.4	4.0	3.8	3.8	4.0	3.9	3.5
Erosion control	3.5	4.0	4.0	3.6	3.7	3.9	3.5
Carbon sequestration	2.5	3.1	2.7	2.2	2.3	3.3	2.7
Mitigating greenhouse gas emissions	3.2	3.7	3.4	3.8	3.3	2.9	3.2
Adaptation to climate change	2.8	4.2	3.0	3.6	3.7	3.2	3.2
Flood plains rivers	3.1	3.9	2.4	2.0	2.7	3.5	3.0
Pathogen control in cropping system	2.7	3.2	3.4	3.4	2.3	2.4	3.2
Fire control	2.7	3.3	2.7	2.4	2.0	2.4	3.3
Avalanche control	1.9	2.8	1.1	1.0	1.3	1.6	2.1

Table 3. Importance of cultural services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Research	Students
Beauty of the landscape	4.1	4.1	4.2	3.6	3.3	4.3	4.2
Positive perception of animal production systems	3.8	3.3	3.7	3.2	2.7	3.6	3.3
Rural development	3.1	3.9	3.3	3.6	2.3	3.2	3.5
Maintaining population in rural areas	2.7	3.8	2.6	2.4	1.7	2.6	3.0
Cultural values	2.5	3.9	3.0	2.8	2.7	3.1	3.6
Tourism / recreation	3.2	3.8	3.1	2.6	3.3	3.7	3.5
Supporting horses for equestrian sport and recreation	2.6	2.9	2.4	1.6	2.7	2.6	2.6

Table 4. Importance of supporting services of grasslands according to the respondents of the questionnaire (1 = not important; 5 = very important).

	Advice	Education	Farmers	Industry	NGO	Research	Students
Grazing	3.8	3.8	3.2	3.6	3.3	3.8	3.3
Animal health	4.3	4.0	4.3	4.6	3.7	4.0	3.8
Animal welfare	4.1	3.4	4.0	3.8	4.0	4.0	3.7
Conservation of soil structure and fertility in cropping systems	3.7	4.0	3.5	3.4	2.3	3.8	3.7
Feed protein supply at farm level	4.4	4.0	4.0	4.0	2.3	3.6	3.3
Competitiveness of farming systems	3.1	3.0	3.2	3.6	3.0	3.0	2.9
N fixation via legumes	3.7	3.6	3.9	3.8	3.0	3.2	3.3
Availability of water	3.7	4.0	3.4	3.6	3.3	3.6	3.8
Crop pollination	3.1	3.0	3.3	2.8	3.0	3.2	3.2

Conclusion

Polish stakeholders consider grassland to be important for a range of functions and services including animal health, dairy cow milk production, nutritional quality of animal products for human consumption, low cost animal feed, beauty of the landscape and biodiversity. The least important functions considered by the stakeholders include goat milk and meat production, wool production, production of plant fibre and avalanche control. All stakeholder groups generally agreed on the importance of the functions evaluated.

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Theme 6 'Approaches to forage crop improvement'

Theme 6 submitted papers

Genomic characterization of survivor populations of red clover by GBS

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Abstract

Survivor populations of red clover from different plots in a field experiment in southern Norway were genetically characterized using genotyping by sequencing (GBS), and compared with the original population and each other. Based on allele frequencies of single nucleotide polymorphisms (SNPs), pairwise genetic distances between populations were calculated for each SNP and across all SNPs as F_{ST}-values and Nei's genetic distance, respectively. Across all SNPs, survivor populations were more different from each other than from the original population, indicating random selection or selection due to local variation in conditions within the site. Fifty-six SNPs were found to have been under selection in all four populations (*FDR=0.016*). These are candidate loci for persistence of red clover.

Keywords: *Trifolium pratense*, genetic shifts, persistence, population genetics

Introduction

Red clover (*Trifolium pratense* L.) is a perennial outbreeding species and thus there is a considerable amount of genetic variation within cultivars. In a field, only a fraction of the seeded plants will survive the first few years due to competition and stress. Surviving plants are presumably genotypes that are best adapted to the local conditions, and may be utilized to identify traits and alleles useful for breeding. The aim of this study was to find out if it is possible to detect loci under selection within one generation of red clover using a GBS approach.

Materials and methods

The diploid red clover cultivar 'Lea' (Graminor, Norway) was included in a split-plot field experiment with pure stands and mixtures of grass and legume species (plot size 7.5 m^2), sown in two replicates at Ås, Norway in June 2010. The plots were harvested either 3 or 5 times a year (3H and 5H) and leaves were sampled once or twice a year. In order to study changes in genetic composition, the original population and survivor populations were genotyped using GBS technology. DNA was extracted from leaves of 48 individual survivor plants sampled in October 2012 from both harvesting regimes in two replicate blocks (four survivor populations). In addition, DNA was extracted from 88 individuals of the original population seeded in the greenhouse. GBS library preparation and sequencing, as well as SNP calling, was done by the Institute for Genomic Diversity, Cornell University, according to Elshire *et al.* (2011). The enzyme ApeK1 was used for digestion of genomic DNA, and the GBS UNEAK analysis pipeline, an extension to the Java program TASSEL (Bradbury *et al.*, 2007), was used to call SNPs from the sequenced GBS libraries. After removing SNPs with missingness >0.2 in one or more of the five populations, or minor allele frequency <0.05 in both the original population and at least one of the four survivor populations, 9203 SNPs remained for analysis. For all these SNPs allele frequencies were calculated for the original population and each of the four survivor populations separately. In order to study the overall differentiation between populations, the average Nei's genetic distance, D (Nei 1972), across all SNPs was calculated for each population pair (Table 1). In order to identify SNP loci that had been under selection, pairwise F_{ST}-values (original population vs. each of the four survivor populations) were first calculated for each SNP as $\frac{\overline{q^2}-\overline{q}^2}{\overline{q}(1-\overline{q})}$. Secondly, a chi-square test was used to identify SNPs with significant

F_{STS} at $P<0.1$, $P<0.05$ and $P<0.01$ levels, using the test statistic $x^2 = 2NF_{ST}$, where $2N$=the sum of genotyped gametes in the two populations (Hedrick 2011). Thirdly, SNPs with significant F_{ST} either in all four survivor populations, in both replicate 3H survivor populations or in both replicate 5H survivor populations, all relative to the original population, were identified. Corresponding estimates of the false discovery rate (*FDR*) were calculated for each *P*-level as $\frac{l*P^n}{d}$, where l=number of SNP loci tested (9203), n=the number of survivor populations tested against the original population (4), and d=the number of SNP loci identified as significant in all n population pairs. Pairwise F_{STS} between the 3H and 5H survivor populations within both replicates were also calculated and tested, but this did not result in any SNPs detected with an acceptable *FDR*. The SNP-containing sequences were blasted against sequences available in the Legume Information System (http://www.comparative-legumes.org/).

Results and discussion

Nei's genetic distance between the original population and the survivor populations were significantly smaller than the distances between survivor populations (Table 1).

Table 1. Nei's genetic distance (D) between populations. D values were first averaged across 9203 SNPs for each population pair and then across 2-6 population pairs.

Compared populations (number of pairwise comparisons)	Average pairwise D ± S.E.
Original population and all survivor populations (4)	0.00351 ± 0.00014
Original population against both 3H survivor populations (2)	0.00363 ± 0.00024
Original population against both 5H survivor populations (2)	0.00339 ± 0.00019
All survivor populations against each other (6)	0.00433 ± 0.00010
3H and 5H survivor populations against each other (4)	0.00443 ± 0.00009
Rep 1 and rep 2 survivor populations against each other (4)	0.00435 ± 0.00014

Also, the distance between 3H and 5H populations were no larger than the distance obtained when populations were grouped according to replicate block instead of harvesting regime. Together, this indicates that random selection or diversifying selection due to variation in local conditions in the field other than harvesting regime has occurred. If the original population has high genetic diversity and low linkage disequilibrium (typical of forage cultivars), it cannot be expected that selection acting on a relatively limited number of loci will affect average genetic distance measured across the genome. In order to identify such selection, genetic distance of individual loci must be considered. Indeed, some loci had been under selection in all four plots: 56 SNPs had significantly altered allele frequencies, measured as F_{ST}, in all four survivor populations relative to the original population (*P*<0.1 all survivor populations, corresponding *FDR*=0.016). Twenty of these were also significant at *P*<0.05 in all survivor populations, with a corresponding *FDR*<0.003. The use of several survivor populations makes it possible to use a less stringent significance level in the first screening of SNPs in individual populations. In addition, effects of random selection are removed. These 56 SNPs are likely selected by conditions that are common to all four plots and can be related to, e.g. establishment, competition, winter survival and the general environmental and management conditions. They may be associated with genes conferring improved survival through genetic linkage or population structure. Blastn hits were obtained for 13 of the 56 SNP sequences. Hundred SNPs had significant F_{STS} (*P*<0.01, *FDR*=0.028-0.046) in at least two survivor populations. The number of SNPs with significant F_{STS} in both 3H populations or in both 5H populations were not larger than the number of SNPs with significant F_{STS} when the survivor populations were grouped according to replicate blocks (data not shown). Thus, no loci under specific selection in response to harvesting regime could be detected. This may have been possible with a higher number of replicate populations, a higher number of sampled survivors per population, or more SNP loci and higher sequencing depth.

Conclusions

In conclusion, it was possible to identify loci that had been under natural selection within one generation in a red clover variety grown in a field experiment for two and a half years. This may be utilized in further identification of genomic regions, genes and alleles conferring persistence in red clover under various field conditions, which again can be utilized in breeding. In this experiment no specific selection in response to harvesting frequency could be detected - this may be a matter of limiting sensitivity of the experimental setup.

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Towards genomic selection in perennial ryegrass genetic improvement

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Abstract

Genomic selection (GS) for crop improvement makes use of genome-wide molecular marker information. GS has proven its value in animal breeding programmes, but its impact in plant breeding is just emerging. The objective of GS is to use genotype data to estimate and predict the breeding value of selection candidates. This can increase the speed of the breeding cycle, reduce the cost and effort of phenotyping, and achieve faster selection of candidates for crossing programmes and production of varieties. Such genomically estimated breeding values (GEBV) are based on models developed in a training population for which both phenotypic and genotypic data are available. The usefulness of GS is determined by the accuracy of GEBV. The size of the training population and the density of molecular marker coverage are key constraints for prediction accuracy. The perennial ryegrass recurrent selection breeding programme at IBERS is based on a relatively small founder population, and lends itself well to a GS approach. We will describe the integration of GS into the existing breeding programme, and present our initial results of prediction accuracy for various traits.

Keywords: *Lolium perenne*, genomic selection, phenotype, genomically estimated breeding value.

Introduction

Perennial ryegrass (*Lolium perenne* L.) is one of the most important forage crops in temperate European livestock agriculture. Its genetic improvement is based on recurrent selection in populations or families with the aim of increasing the frequency of beneficial alleles or genes over successive generations. Recent developments in genomics technology have made genetic marker development much more cost effective. Genome-wide markers now has the potential to assist the breeding programmes by providing accurate information of estimated breeding values faster than phenotypic information. Genomic selection (GS) was first proposed by Meuwissen *et al.* (2001), and is now widely used in animal breeding programmes (Hayes *et al.*, 2013). The potential of GS in plant breeding has now also been recognised (Heffner *et al.*, 2009). The advantage of GS compared to traditional marker-assisted selection is that the effects of all markers are used simultaneously, irrespective of whether they are significant or not.
Implementation of GS requires a reference or training population for which both phenotypic data and information of evenly distributed molecular markers are available. This information is used to generate prediction models to estimate the effects of each marker, and used to estimate the breeding value for plant material in test populations with genotypic data, but for which no phenotypic information is available. This is potentially advantageous for difficult or expensive to measure traits, and in perennial crops with long generation cycles. We are using the IBERS forage ryegrass breeding populations as templates for implementing GS. Here we describe the important features of this project, provide preliminary data on prediction models and their accuracy, and discuss future improvements.

Materials and methods

Phenotypic data for a range of traits were initially obtained from plot trials performed on half-sib progeny of mother plants from the breeding programme. Two breeding populations were used: the 13th generation of the intermediate flowering population, and the 5th generation of the late flowering population. This provided the first training population, and had a combined size of 159. An Illumina Infinium SNP-CHIP with single nucleotide polymorphisms from 2765 genetic loci was used to obtain the genotypic data from this population. In addition 100 motherplants from the 14th intermediate generation were also genotyped. They constituted the test population. We used a genomic best linear unbiased prediction model (GBLUP) to provide us with marker effects and GEBVs from the training population. Due to a relatively small size of the two populations, model fit was assessed by leave-one-out cross validation, in which prediction for each sample point was obtained by using a model fitted to the remainder of the data excluding that point. Accuracies were estimated by calculating the mean squared error of the cross-validated predictions and dividing it by the sample variance. This way one can compare performance of the BLUP model to that of the 'base' model that predicts every point with the sample mean.

Results and discussion
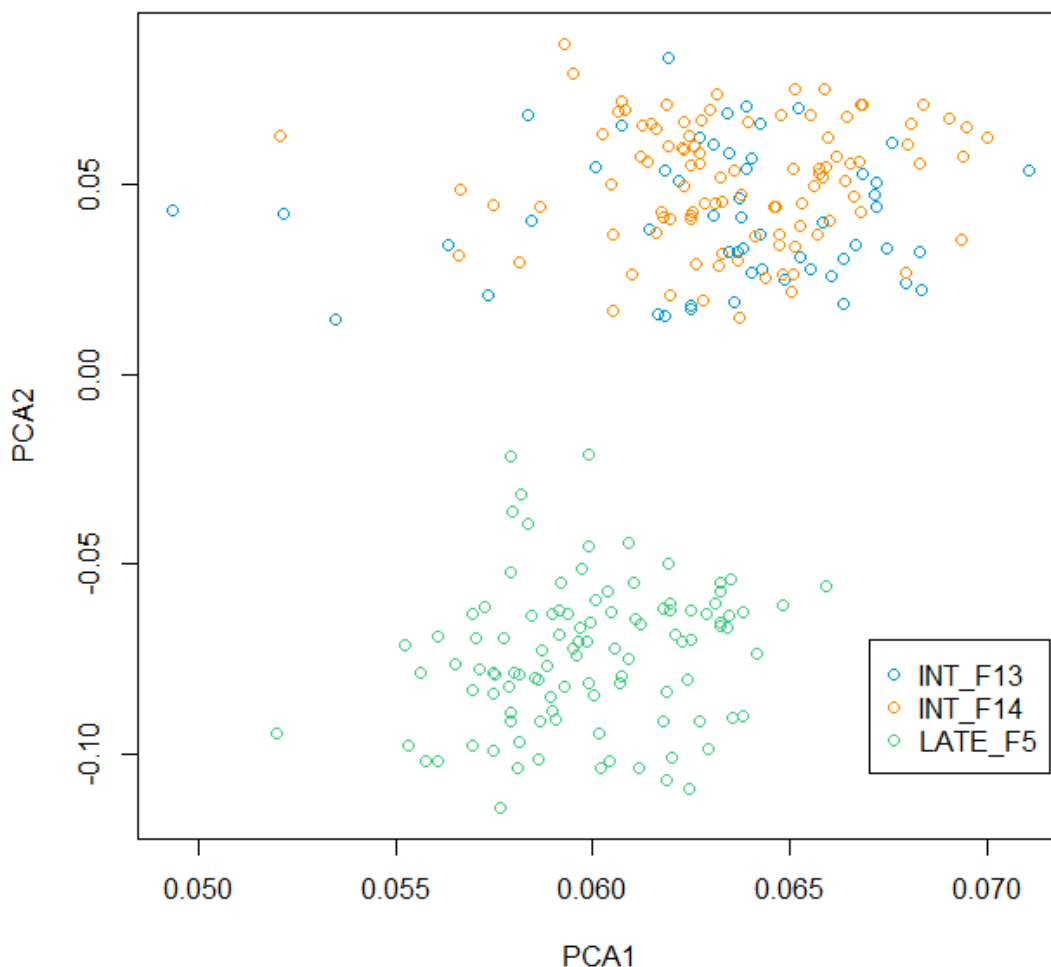
Figure 1. Principle component analysis of motherplants from two generations of the intermediate flowering and the late flowering populations. The analysis is based on marker data information.

The late and intermediate flowering populations differ both at the genotypic and phenotypic levels. Principal component analysis separates Intermediate (F13 and F14) and Late (F5) genotypes into two clearly defined clusters (Figure 1). This suggests that the F13 data would be most useful in predicting the F14 breeding values.

The traits possess large variability across years as well as across the two populations: some traits, like dry matter digestibility (DMD), are more or less homogenous across the two sub-populations, while most, like yield, vary considerably. Additionally some traits are highly correlated, e.g. DMD and water soluble carbohydrate (WSC) and yields in the two consecutive years.

We analysed the two populations separately and combining the two into a larger pooled sample. Results for GBLUP are summarized in Table 1. The BLUP model performs rather poorly for some of the traits with no difference from the 'base' model and with little, if any, accuracy gain by using the pooled populations, but explains some of the variance in the others. Notably, it is the forage quality traits which are more homogenous across the two sub-populations that benefit from using the pooled sample.

Table 1. Broad sense heritability (H^2) and prediction accuracies for traits measured in the perennial ryegrass breeding populations. A low prediction value indicates a good model performance, a value close to 1 indicates no difference from 'base' model. gcscore – ground cover, DMD – dry matter digestibility, WSC – water soluble carbohydrates, N – nitrogen, DMyield2 – yield of 2nd cut, vegyld – yield of the other cuts, NDF_dig – neutral detergent fibre digestibility.

Test	Int F13	Int F13 – Prediction		Late F5	Late F5 – Prediction	
Train	$H^2 \pm SD$	Int F13	Pooled	$H^2 \pm SD$	Late F5	Pooled
gcscore_yr3	0.51±0.97	1.03	1.05	0.29±9.37	0.93	0.92
yield_yr1	0.71±0.39	1.01	0.99	0.00±0.00	1.02	1.04
yield_yr2	0.67±0.37	0.80	0.70	0.57±0.60	1.03	0.96
DMD	0.82±0.10	0.69	0.63	0.39±1.28	0.96	0.92
WSC	0.73±0.21	0.79	0.71	0.61±0.52	0.86	0.82
N	0.67±0.33	0.94	0.74	0.51±0.52	0.85	0.81
DMyield2_yr1	0.00±0.00	0.99	1.02	0.36±1.10	1.07	1.03
DMyield2_yr2	0.00±0.00	1.04	0.91	NA	0.89	0.86
vegyld_yr1	0.76±0.20	0.96	0.90	0.28±119	0.86	0.87
vegyld_yr2	0.67±0.33	0.82	0.75	NA	1.04	1.03
NDF_dig	0.55±2.01	0.75	0.66	0.58±0.58	0.88	0.85

The BLUP model is essentially a ridge regression, so no attribute selection is performed. With over 2700 markers this invariably causes overfitting and hence reduced prediction accuracy. Indeed, machine-learning techniques (regression trees, lasso regression) achieve slightly better results for some traits (e.g. vegyld) but make no difference in others. This suggests that larger data set (more markers as well as more plants) is required in order to fit more successful models. Nevertheless, GEBV of the motherplants in the Late-flowering 5th population were able to accurately predict three of four parents selected for variety production based on an overall merit phenotypic evaluation.

Conclusions

A larger training sample and more markers are required to fit models capable of uncovering links between phenotypic traits and genotypic data. We hope to achieve this by adding historic data as well as new data from existing generations. Moreover, a new SNP-CHIP will increase the number of markers to around 4000. Coupled with powerful machine learning techniques, capable of attribute selection as well as uncovering non-linear relationships between response and regressors, we hope to find markers important for each trait and thus achieve higher prediction accuracies.

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Prospects for introducing genomic selection into forage grass breeding

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Abstract

During the last decade, Genomic Selection (GS) has led to significant genetic progress in animal breeding. Only recently has the potential of GS in plant breeding been discussed. This paper presents the first results of GS implementation in a ryegrass (*Lolium perenne* L.) breeding programme. Both phenotype and genotype information were collected from 990 F2 families produced within a standard breeding programme at DLF-Trifolium. Phenotype data were recorded as family means, while genotype data were expressed as family allele frequencies. Statistical analyses were performed using linear mixed models (GBLUP), and displayed the presence of a significant level of genetic variance in all traits that was almost fully explainable by the genomic relationship matrix. Preliminary cross-validation analyses on heading date and crown rust resistance showed reliabilities around 0.50. Further studies will be undertaken on model development and in the study of gene by environment interactions.

Keywords: Genomic Selection, *Lolium perenne*, GBLUP, GxE

Introduction

Genomic Selection (GS) is a form of Marker Assisted Selection (MAS), which explains the total additive genetic variance and calculates the individual's Genomic Estimated Breeding Value (GEBV) by simultaneously estimating the effects of all loci across the entire genome. GEBVs are calculated from genome wide markers of sufficient density to ensure all loci are in Linkage Disequilibrium (LD) with at least one marker. This has been most commonly achieved by genotyping single individuals with high-density hybridization-based arrays. GS is now well established in human medicine and animal breeding, mainly in cattle and pigs. In plants, it is expected to considerably speed up the genetic progress, resulting in a decrease of the progenies needed to obtain a certain level of gain, especially for complex traits with low heritability (Bernardo, 2008). However, so far it has been tested almost exclusively through simulations and its actual potential has yet to be evaluated in reality (Conaghan and Casler, 2011). The aim of this study was to explore the possibility for implementation of GS in perennial ryegrass breeding. This faces two major challenges: (i) phenotypes recorded on heterogeneous families, (ii) high heterozygosity, which makes genotypes calls difficult on hybridization-based arrays. This work shows the methodologies used to overcome such problems, together with the first preliminary results.

Materials and methods

Plant material: 990 F2 families, produced between 2000 and 2009 in a standard breeding programme run by DLF-Trifolium.
Phenotypic data: all families were sown, in multiple plots, in seven locations across Europe in the production year, and in Bredeløkke (DK), in 2011. Plots were then farmed for two cropping seasons. Data on different agronomic traits have been collected: (i) forage yield (green [GMY] and dry matter yield [DMY]), measured in kg m^{-2}, (ii) scored traits, measured with a scale from 9 to 1: heading date, aftermath heading, crown rust resistance, winter hardiness, density, spring

growth. All traits were scored once on each replicate, except for crown rust resistance, which was scored three times per year.

Genotype data: Genotype-By-Sequencing (GBS) libraries were prepared from individually barcoded F2 families. Sequencing data were aligned to a draft assembly of the *L. perenne* genome and SNPs were identified within the population. Allele frequencies of the variant were calculated at each SNP position.

Statistical models: preliminary analyses were conducted using the following Linear Mixed Model: $y = X_1t + Z_1i + Z_2p + Z_3i_l + Z_4p_l + Z_5c + e$, where: X_i, Z_i = design matrices for fixed and random effects; t = vector of trial within year and location; i = vector of breeding values; p = vector of parent populations (pps); i_l, p_l = vectors of breeding values and pps within location; c = vector of environmental effects within trial; e = residuals. In the model for crown rust resistance, the effect "scoring time" and its interaction with the other variables were added. Variance components were estimated by Restricted Maximum Likelihood algorithm (REML) using the software DMU (Jensen *et al.*, 1997; Madsen and Jensen, 2010). Heritabilities were expressed as 'within' and 'between' locations. Genomic information, based on family allele frequencies, was then implemented by GBLUP, which use the genomic relationship matrix (G-matrix) as (co)variance structure of the breeding values. Preliminary analyses were performed for heading date and crown rust resistance. Prediction reliabilities were determined by cross-validation, deleting one family at the time from the dataset.

G-matrix: It summarizes the inheritance of multiple phenotypic traits. It was constructed in the following way (Legarra and Misztal, 2008; Vanranden, 2008; Forni *et al.*, 2011): given a matrix S, comprised of allele frequency estimates, X_i being the allele frequencies of a certain family and M being the centred matrix $M_i = X_{ij} - \overline{X_i}$, with $j = 1,2,...990$, G-matrix is equal to: $G = (M'M)/K$. K is a scaling parameter (K = Average diagonal ($M'M$)). Different G-matrices were obtained by selecting SNP markers with different sequencing depth and used for analyses.

Results and discussion

Variance components: All traits showed a significant level of genetic variance (Table 1).

Table 1. Heritabilities in the historical data (with SE) across locations (-A) and for one location (-L). GMY = green matter yield; DMY = dry matter yield.

Character†	h^2_A	h^2_L
GMY	$0.20_{0.026}$	$0.65_{0.022}$
DMY	$0.30_{0.030}$	$0.57_{0.029}$
Aftermath heading	$0.34_{0.046}$	$0.59_{0.033}$
Winter hardiness	$0.16_{0.049}$	$0.64_{0.042}$
Density	$0.17_{0.072}$	$0.59_{0.057}$
Crown rust resistance	$0.26_{0.033}$	$0.37_{0.022}$
Spring growth	-------	$0.48_{0.031}$
Heading date	-------	$0.67_{0.052}$

For heading date and spring growth, it was not possible to estimate heritabilities within location, due to lack of repeated plots. Heritabilities across locations ranged between 0.17 and 0.34, depending on the trait. Estimates at one location were always higher (up to 0.67), showing the presence of significant Gene × Environment (G×E) interactions. A remarkable exception was crown rust resistance. In this case, the larger level of variation occurred between scorings, rather than between locations. Results also showed correlations between first-cut yield, heading date and aftermath heading (around 0.30), with early heading plant showing highest yield in spring and increased stem production.

GBLUP and cross-validation: The genomic relationship matrices were able to explain a high proportion of the genetic variance. Results from both simulated and real data indicate genomic variance to be overestimated at low sequencing depth. That is caused by inflated diagonals in the genomic relationship matrix based on sequence data and, consequentially leads to an underestimation of the remaining family effect. There is no impact of sequencing depth on the estimate of environmental variances. Preliminary results from cross-validation showed reliabilities of GEBVs (squared correlation between GEBV and true breeding value) around 0.50 for heading date and crown rust resistance.

Conclusions

The amount of genetic variance, the proportion of variance explained by the G-matrix and the first results from cross-validation appear promising for implementation of GS in the breeding programme. Further studies are needed to take account of low sequencing depth. Different models (Bayesian Lasso, Bayesian variable selection) will be tested on several traits, to identify potential genes of large effects and estimating the effect of G×E interactions. Results for crown rust resistance seem to show a potential for developing varieties, which perform well across locations.

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Population selection within perennial ryegrass cultivars under simulated grazing

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Abstract

Perennial ryegrass (*Lolium perenne* L.) sward populations have the potential to change as they age, either positively or negatively depending on the synergy with the environment where they are sown. Although previous studies have examined population selection, few have examined an array of perennial ryegrass genotypes. The objective of this study was to examine if sward populations became physiologically unique from that at sowing as a result of sward management. Genotypes from 12 cultivars managed under simulated grazing for 5 years (A) were compared to a control sown from breeders seed of the same cultivar (C), creating 24 accessions which were sown as spaced plants. Plants were subjected to a number of phenotypic measurements as described by the Distinctness, Uniformity and Stability (DUS) tests (UPOV, 2006). Control accessions were distinguishable from each other except for 2. Aged accessions where distinguishable from their C accession equivalents, except for 1. Mean date of inflorescence emergence was the most useful character in distinguishing C accessions, while natural height a mean date of inflorescence emergence was the most useful character in distinguishing between A and C accessions.

Keywords: perennial ryegrass, population selection

Introduction

Perennial ryegrass cultivar populations can have large genetic variation within population (Guthridge *et al.*, 2001). As sward genotypes age, the genetic variance of the remaining population will reduce from the initial sowing date (Charles, 1970). This creates the opportunity for the characteristics of the sward to change over time, potentially negating the positive characteristics of a newly established cultivar and may be influenced by both environmental and management factors. Charles (1970) found selection occurred for both a higher yielding population and a lower yielding population in two separate swards compared to the original breeders seed. In a more recent study, selection for later mean date of inflorescence emergence and smaller plant height within a grazed cultivar was noted (Hazard *et al.*, 2006). Previous investigations into population selection have examined a small number of cultivars of perennial ryegrass. Due to the large variation between perennial ryegrass cultivars, it is unclear how cultivar choice will influence population change. The objective of this study was to examine if the accessions could be described as distinct 'varieties' and to determine if population change occurred across a wide range of perennial ryegrass cultivars.

Material and methods

Plant populations for twelve cultivars (4 diploids (D) and 8 tetraploids (T)) were established as spaced plants, with two treatments for each cultivar, creating 24 accessions. The cultivars and their heading dates were: Alto (D; 15 May), Arrow (D; 22 May), Bealey (T; 22 May), Dunloy (T; 8 June), Dunluce (T; 31 May), Glencar (T, 6 June), Greengold (T; 31 May), Lismore (T; 28 May), Malone (T; 22 May), Navan (T; 9 June), Portrush (D; 14 June), Tyrella (D; 8 June). The two treatments were: aged (A) and control (C). The A accessions were sown as plots in autumn

2006. Plots were arranged in a randomized block design with three replicates. Each plot was 5 m × 1.5 m (7.5 m²). The plots were mechanically defoliated using a motor agria to a height of 4 cm (Etesia UK Ltd., Warwick, UK) from 2007 to 2011, inclusive. The aged plots were defoliated 10 times per year for the duration of the experiment. In September 2011, 35 perennial ryegrass tillers were extracted from each of the plots and sown in multi-pot trays, creating a total of 105 collected tillers for each A accession. The location from which each tiller was extracted was evenly spread across the plot, while avoiding the boundary areas. At the same time, plants were established in multi-pot trays from seed. The C accessions were derived from seed of the original seed lines for each of the twelve cultivars. Plants were housed in a glasshouse over winter before been placed outside in the trays in spring 2012. Tillers were cut with a shears every 4 weeks and fertilized to maintain healthy plants and promote tillering. In August 2012, a ley area was ploughed and cultivated. Plants were sown in a randomized block design with eight replicates per accession and ten spaced plants per replicate. Each plant was spaced 0.75 m from each of the plants surrounding it. A row of non-experimental guard plants were sown around the perimeter of the experiment to negate boundary effects on plants included for measurement.

Plants were subjected to a number of phenotypic measurements as described by the Distinctness, Uniformity and Stability (DUS) tests (UPOV, 2006). Full details of the 22 characters recorded are described in the DUS protocol, the subset listed below presents the most important characters within the current experiment. Plants were checked for inflorescences emergence every Monday, Wednesday and Friday until inflorescences emergence had been observed on all plants. Mean date of inflorescences emergence was recorded when 3 ears had visibly emerged beyond the ligule on an individual plant. On the mean date of inflorescence emergence, natural plant height at inflorescence emergence was recorded. Finally, inflorescence length was measured with a ruler 30 days after the mean date inflorescences emergence for each accession replicate. Analysis was conducted using DUST9, SUMM9 and ANAL9 modules of the DUST analysis system (Weaterup, 1998).

Results and discussion

In the current experiment all the control accessions were distinct from each other except for Dunluce and Greengold (Table 1). The large number of distinctions between the control accessions confirms that the experiment was successful in discrimination between accessions that are deemed to be distinct on the European common catalogue of perennial ryegrass varieties (European Commission). Mean date of inflorescences emergence and inflorescence length created the most distinctions between control accessions, separating 77% and 59% of the total pairs tested, respectively.

In all genotypes except Navan, the C accessions were distinct from their A equivalents (Table 1). The greatest number of distinct characters was observed between the C and A accessions within the Glencar and Greengold genotypes with 14 of characters distinct for both genotypes. In contrast, between the C and A accessions within the Alto and Lismore genotypes, only 1 character was distinguishable. Natural height at inflorescence emergence created the greatest number of distinctions between C and A accessions within genotype, accounting for 58% of pairs separated. A reduction of 23% for natural height at inflorescence emergence was observed from the C to the A accessions. Mean date of inflorescence emergence was the only character that did not create any distinctions between C and A accessions within genotype. This indicates that perennial ryegrass genotypes change over time in a simulated grazed sward and genotypes can change independently of each other. These results show a distinct change in population but will require a second year of measurement in order to be confirmed. The change in cultivar population may partly explain a change in the trait expression of cultivar leys over time.

Table 1. (1) Number of characters distinct between control genotypes, and (2) between control and aged accessions within genotypes

Genotype	Alto	Arrow	Bealey	Dunloy	Dunluce	Glencar	Greengold	Lismore	Malone	Navan	Portrush	Aged
Alto												1
Arrow	3											2
Bealey	5	6										5
Dunloy	17	17	14									10
Dunluce	17	16	13	1								12
Glencar	15	17	10	2	2							14
Greengold	15	14	12	1	0	2						14
Lismore	17	18	15	1	1	4	2					1
Malone	7	9	3	5	2	4	1	8				3
Navan	13	15	12	4	3	5	2	2	4			0
Portrush	12	12	15	8	9	11	7	9	9	6		7
Tyrella	6	9	11	12	13	10	7	15	6	9	4	3

Conclusion

With one year of measurement in this experiment it was possible to distinguish between all but two of the control accessions. All genotypes except Navan changed over time; however, there was a marked difference in the extent of character change observed between genotypes tested, with a large amount of characters distinct between certain accession pairs compared to very few distinct between other accession pairs. Mean date of inflorescence emergence was the most useful character when discriminating between C accessions. The characters that were most powerful in distinguishing between C accessions were not the most useful in determining distinctness between the C and A accessions within genotype. Natural height at inflorescence emergence proved to be the most useful character in distinguishing between C and A genotypes. Population selection of perennial ryegrass cultivars has the potential to change the trait expression of cultivars over time.

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Genetic gain in yield of perennial ryegrass (*Lolium perenne*), Italian ryegrass (*Lolium multiflorum* Lam.) and hybrid ryegrass *(Lolium x boucheanum Kunth)* cultivars in Northern Ireland Recommended Lists 1972-2013

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Abstract

Ryegrass cultivars form the basis of grassland production in Ireland and the UK. In Ireland, perennial ryegrass (*Lolium perenne)* is the dominant ryegrass species followed by Italian ryegrass (*Lolium multiflorum* Lam.) and Hybrid ryegrass (*Lolium x boucheanum* Kunth). Such cultivars are selected for their high dry matter (DM) yield throughout the year. However, there is a knowledge gap regarding the genetic gain achieved in DM yields across ryegrass cultivars under Irish conditions in recent years. Data were obtained from testing carried out for the Recommended List in Northern Ireland on the DM yields of ryegrass species for 1973-2012 for perennial, 1972-2013 for Italian and 1974-2012 for hybrid ryegrass. Each cultivar was tested under a simulated grazing and conservation management for a minimum of three years. Cultivars were grazed by animals for each management in the first year. For the simulated grazing management, 320 kg N ha^{-1} were applied and there were seven harvests to a residual height of 3 cm. For the conservation management, 350 kg N ha^{-1} were applied and there were five harvests to a residual height of 6 cm over the following two years. Italian and hybrid ryegrass were subjected to a 3-cut conservation management receiving 425 kg N ha^{-1} Genetic gains in DM yield have been achieved in perennial (+0.43%/year), Italian (+0.37%/year) and hybrid ryegrasses (+0.26%/year).

Keywords: *Lolium perenne, Lolium multiflorum* Lam., *Lolium x boucheanum* Kunth, genetic gain, DM yield

Introduction

Perennial ryegrass (*Lolium perenne* L.), Italian ryegrass (*Lolium multiflorum* Lam.) and hybrid ryegrass (*Lolium x boucheanum* Kunth) are the dominant forage grasses in north-western Europe (Wilkins and Humphreys, 2003). A major focus of ryegrass breeding has been to achieve high levels of annual herbage production. However, rates of genetic gain in ryegrass cultivars are not well documented. Previous estimates have reported gains in DM yield of 4-5% per decade in perennial ryegrass (PRG) since the 1970s in Europe (Wilkins and Humphreys, 2003) with similar rates of gain reported in New Zealand (Easton *et al*., 2002). It is known that cultivars can re-rank when managed under a simulated grazing management when compared to a conservation management (Wims *et al.,* 2009). Therefore the rate of gain under both simulated-grazing and conservation managements needs to be established. Furthermore, there is little information on the rate of genetic gains in the dry matter (DM) yield of hybrid and Italian ryegrass cultivars. The aim of this study is to evaluate the rate of genetic gain in the DM yield of perennial, Italian and hybrid ryegrass cultivars listed on the Northern Ireland recommended lists over the past 40 years. The data obtained from the Northern Ireland recommended list provide a unique opportunity to evaluate gains in DM yield, as all cultivars were managed under a consistent protocol, at the same site, and under low disease or environmental pressure.

Materials and methods

Data were obtained on the DM yield of perennial, Italian and hybrid ryegrass cultivars from the Crossnacreevy Plant Testing Station, Co. Down, for the period of 1973-2013. These data were primarily used to compile the Northern Ireland recommended lists of grass cultivars. The dataset comprised 205 (40 early, 88 intermediate and 77 late-heading) perennial ryegrass cultivars. The dataset also included 35 Italian and 27 hybrid ryegrass cultivars. All cultivars were tested for a minimum period of three years under the recommended list protocol (DARD, 2013). In the first year, cultivars were grazed with cattle and measurements were then taken over the next two years under both a simulated grazing management and a conservation management. Perennial ryegrass was subjected to a simulated grazing management comprising seven harvests to a residual height of 3 cm and applications of 320 kg N ha^{-1}. The conservation management comprised five harvests to a residual height of 6 cm and applications of 350 kg N ha^{-1}. Italian and hybrid ryegrass were subjected to a 3-cut conservation management receiving 425 kg N ha^{-1}. Data were analysed using fitted constant analysis to create comparable over-years mean values for each variety.

Results and discussion

Results from the study are presented in Table 1. For perennial ryegrass higher rates of genetic gain have been achieved under conservation management (+0.51%/year). Longer re-growth intervals under conservation management may allow cultivars to express more of their genetic potential. Higher rates of genetic gain in DMY have been achieved in perennial (+0.43%/year) and Italian (+0.37%/year) compared to hybrid ryegrass (+0.26%/year). Perennial ryegrass is by far the most important of these three species in terms of seed sales, accounting for 95% of seed sales in Ireland (Culleton *et al*., 1992). This would suggest a larger breeding effort on perennial ryegrass cultivars by plant breeders resulting in greater gains in DM yield. Within perennial ryegrass there have been slightly higher rates of genetic gain in early- (+0.46%/year) and late-heading cultivars (+0.45%/year) in comparison to intermediate- (+0.39%/year) heading groups. Increase in late-heading performance maybe attributed to a greater uptake of late-heading cultivars on farms (Grogan and Gilliland, 2011).

These results indicate that the rate of genetic gain in the DM yield of ryegrasses is significantly lower than has been achieved for maize (2.6%/year; Tollenaar, 1989) and wheat (1.0%/year; Calderini *et al*., 1995). This may be due to the greater difficulties encountered in breeding ryegrasses. For example, there is not much scope for altering the harvest index of grasses, whereas much of the improvement in the grain yield of cereal crops has been achieved by increasing the proportion of plant biomass allocated to grain (Wilkins and Humphreys, 2003). There is also genetic variation within a ryegrass population making selection for a specific trait more difficult. Furthermore, the higher seed sales of cereal crops allow greater financial investment in plant breeding.

Conclusion

This study demonstrated that gains in DM yield have been achieved over the last 40 years across ryegrass species with the exception of hybrid ryegrass. There have been greater gains achieved in conservation yields of ryegrass cultivars. However, gains achieved are not significantly greater in comparison with other estimates in Europe. This highlights that further research is required to investigate possible pathways to improve the rates of genetic gain in ryegrass breeding.

Table1. The percentage of annual gains in dry matter yield achieved in perennial ryegrass (1973-2013), Italian ryegrass (1972-2013) and Hybrid ryegrass (1974-2012) for early-, intermediate- and late-heading varieties from the Northern Ireland Recommended List, under simulated grazing and conservation management.

Grass species and heading-date groups	No. of Cultivars	% gain per year	Gain in total DM yield t ha^{-1}	P- value
Perennial ryegrass				
Simulated yield	202	+0.35	+1.6	***
Conservation yield	199	+0.51	+2.8	***
Heading group				
Early				
Simulated yield	40	+0.44	+2.0	***
Conservation yield	39	+0.48	+2.7	***
Intermediate				
Simulated yield	88	+0.26	+1.2	***
Conservation yield	86	+0.52	+2.9	***
Late				
Simulated yield	75	+0.4	+1.8	***
Conservation yield	73	+0.5	+2.8	***
Italian ryegrass				
Conservation yield	35	+0.37	+2.5	***
Hybrid ryegrass				
Conservation yield	27	+0.26	+1.0	NS

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Variation in the reproductive development of perennial ryegrass (*Lolium perenne*) cultivars

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Abstract

Plant breeding has manipulated the flowering of perennial ryegrass (*Lolium perenne*) by developing later heading cultivars. However, the impacts of breeding on the intensity and temporal distribution of flowering are not known. This study compared the reproductive development of 23 perennial ryegrass/endophyte combinations. Two replicate plots were closed from grazing and tillers were collected fortnightly over a 10-week period, commencing on 21 October 2013. Plant development stage was determined on a sub-sample of 30 individual tillers per replicate, according to the Moore *et al.* (1991) scale. The development of each cultivar was then calculated using the mean stage count formula of Moore *et al.* (1991). The rate and timing of reproductive development differed among cultivars. As expected, cultivars with mid-season heading dates matured earlier than cultivars classified as late- and very late heading. While the intensity of flowering was similar between maturity groups, the temporal distribution of reproductive development varied: the late- and very late-season maturing groups had lower proportions of reproductive tillers early in the season.

Keywords: *Lolium perenne*, reproductive development, flowering behaviour

Introduction

The nutritive value and growth pattern of perennial ryegrass (*Lolium perenne*) in spring and summer is influenced by its flowering behaviour. Nutritive value declines as plants move from vegetative growth to reproductive growth in spring (September to November in the southern hemisphere), in part due to changes in plant morphology. Plant breeding has manipulated the flowering of perennial ryegrass by selecting for later-heading cultivars that maintain their leafiness for longer and retain better nutritive value in spring (Lee *et al.*, 2012). There are now ryegrass cultivars available with a range of heading dates. For example, in New Zealand perennial ryegrass cultivar heading dates range from -17 to +25 days (with day '0' typically 22 October). However, the impacts of breeding on the intensity and temporal distribution of flowering are not known. The objective of this study was to compare the reproductive development of 23 perennial ryegrass cultivar/endophyte combinations spanning four decades of plant breeding in New Zealand.

Materials and methods

The reproductive development of 23 perennial ryegrass cultivar/endophyte combinations was compared in a pasture field trial in the Waikato, New Zealand. Cultivars included mid- and late-heading diploids, and late- and very late-heading tetraploids with a 25-day range in nominal heading date. Based on their nominal heading date, cultivars were classified into one of three maturity groups: mid-season maturing (day 0 to +6), late-season maturing (day +7 to +21) and very late-season maturing (day +22 to +25). Two replicates plots were closed from grazing and from late spring to early summer (21 October to 16 December 2013), tillers were collected fortnightly over a 10-week period. Plant development stage was determined on a sub-sample of 30 randomly selected individual tillers per replicate, according to the Moore *et al.* (1991) scale. A numerical index was then applied allowing the development stage of each cultivar to be calculated which was expressed as an Adjusted Mean Stage Count value (Moore *et al.*, 1991).

The proportion of vegetative, elongating and reproductive tillers was also determined on both a numerical and dry weight basis. Data were analysed using a mixed models approach to repeated measures analysis of variance (Proc Mixed, SAS 9.3) with maturity group, sampling event, and their interaction as fixed effects, followed by a Tukey's test for pairwise comparisons.

Results and discussion

The pattern of reproductive development differed between maturity groups. As expected, cultivars with mid-season heading dates matured earlier, while cultivars classified as late- and very-late heading matured later in the season. Figure 1 presents the adjusted mean stage count for each maturity group through the experimental period.
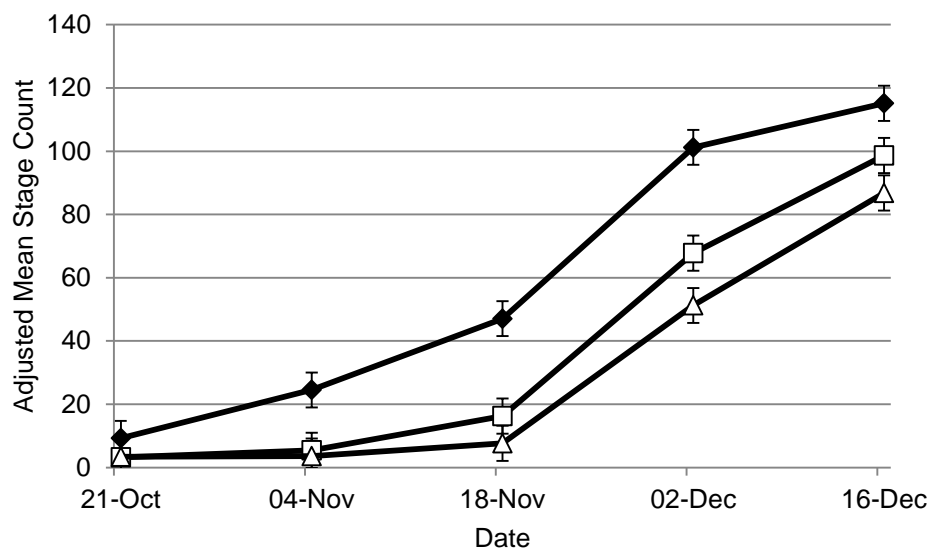
Figure 1: The adjusted mean stage count (± standard error of the difference) of mid-season maturing (♦), late-season maturing (□) and very late-season maturing (Δ) cultivars over five successive sampling events in late spring to early summer

A maturity group by sampling date interaction ($P<0.001$) was observed as the rate of reproductive development differed between the maturity groups. Initially the rate of reproductive development was greater for the mid-season maturing cultivars, while during the final sampling period the rate of reproductive development was greater for the late- and very late-season maturing cultivars. At the final sampling event, the proportion of tillers that had entered reproductive development was greater ($P<0.05$) for the mid-season maturing cultivars compared to the late- and very late-season maturing cultivars (0.76 versus mean 0.65 respectively; Table 1). However, at the final sampling, the proportion of reproductive tillers on a mass basis was similar between maturity groups (mean = 0.88), which may be more meaningful as tillers accumulate more dry matter as they mature (Moore *et al.,* 1991). This suggests that the intensity of flowering was similar for each maturity group, although an additional sampling period would have been valuable to confirm this. While from an animal performance point of view cultivars that have substantially less flowering are highly desirable, this result is perhaps not surprising, as cultivars must have sufficient seed yield to be commercially successful (Stewart and Hayes, 2011).

Table 1: Reproductive tillers in the mid-season maturing (Mid), late-season maturing (Late) and very late-season maturing (VLate) cultivars expressed as a proportion of total tiller number or total tiller mass in each sample.

	Number					Mass			
Sampling	Mid	Late	VLate	$P <$	Sampling	Mid	Late	VLate	$P <$
21 Oct	0.01	0.00	0.00	NS	21 Oct	0.01	0.00	0.01	NS
4 Nov	0.17[a]	0.00[b]	0.00[b]	0.001	4 Nov	0.33[a]	0.01[b]	0.00[b]	0.001
18 Nov	0.39[a]	0.07[b]	0.01[b]	0.001	18 Nov	0.63[a]	0.14[b]	0.03[c]	0.001
2 Dec	0.77[a]	0.58[b]	0.43[c]	0.001	2 Dec	0.93[a]	0.79[b]	0.64[c]	0.001
16 Dec	0.76[a]	0.66[b]	0.63[b]	0.05	16 Dec	0.93	0.86	0.85	NS
Significance		$P <$			Significance		$P <$		
Maturity		0.001			Maturity		0.001		
Sampling		0.001			Sampling		0.001		
Maturity × Sampling		0.001			Maturity × Sampling		0.001		

Not Significant; [a,b,c] Means within a row with different superscripts differ ($P<0.05$)

The temporal distribution of reproductive development varied between the maturity groups; following the initial sampling on 21 October, where there were virtually no reproductive tillers present, a greater proportion ($P<0.001$) of reproductive tillers was recorded for the mid-season maturating cultivars between 4 November and 2 December on both a numerical and mass basis. Reproductive development in spring leads to a decline in pasture quality in part due to an increasing proportion of stem to green leaf. To lessen the effects of reproductive development on pasture quality, plant breeding has focused on delaying flowering in spring by developing later heading cultivars. Results from this study suggest that selection for later-heading cultivars indeed leads to fewer reproductive tillers during spring, which may be promising from an animal performance perspective. However, evaluating the impact of altered flowering behaviour on pasture nutritive value is difficult due to a lack of primary data. Further investigation to evaluate the impact of later heading cultivars on pasture nutritive value and animal performance in seasonal pasture-based livestock production systems in New Zealand would be valuable.

Conclusions

Selection for later-heading cultivars in New Zealand has altered the temporal distribution of reproductive tillers: late- and very late-season maturing cultivars maintain lower proportions of reproductive tillers during mid-spring. There was little evidence to suggest that plant breeding has altered the flowering intensity of perennial ryegrass cultivars.

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Pasture profit index: updated economic values and inclusion of persistency

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Abstract

A pasture profit index was developed to identify the economic merit of a perennial ryegrass (*Lolium perenne* L.) cultivar for a grass-based ruminant production system. The traits of importance were: spring, mid-season and autumn DM yield, 1st- and 2nd-cut silage DM yield, quality (per unit change in DMD/ kg DM) across the months of April to July (inclusive), and persistency (relative to a 10-year base). Persistency was quantified by determining the change in ground score (GS) across years. Each 1-unit decline in GS is associated with a 1683 kg loss in DM yield. The use of the grass economic index enables the identification of cultivars that will provide the greatest economic contribution to a ruminant grazing system. The index illustrates the strengths and weaknesses of individual cultivars and it is expected that it will encourage increased usage of the superior performing cultivars.

Keywords: economic index, perennial ryegrass cultivar, DM yield, quality, persistency

Introduction

In Europe, since 1965, grass breeders have increased DM yield by approximately 0.5% per year (Chaves *et al.,* 2009). Clear improvements in breeding cultivars with delayed heading, less secondary heading, increased persistency and improvements in grass digestibility have been less evident. McEvoy *et al.* (2011) introduced the concept of applying economic values to a set of traits that have been identified as the most economically important within grass-based production systems in order to determine the total economic merit of a cultivar. The key traits of importance in an Irish seasonal grass-based production system are: spring, mid-season and autumn DM yield, 1st- and 2nd-cut silage DM yield, grass quality from April to July inclusive, and grass cultivar persistency. When the index was applied to cultivar data, persistency was not included in the overall ranking indices reported by McEvoy *et al.* (2011) due to a lack of information on cultivar persistency. Monitoring the rate of change in GS and linking this to yield decline presents an opportunity to estimate the potential persistency of a cultivar over a period of time. Cultivar evaluation trials are generally conducted for a relatively short period of time (<3 years) whereas at farm level, cultivars are expected to persist for up to 10 years and often longer. This anomaly creates the requirement to allow long-term cultivar persistency to be predicted based on short-term trial performance. The objective of this study was revise the economic values associated with the key traits using up-to-date price and cost information, while including persistency in the total economic merit of a cultivar.

Materials and methods

Sixty-three perennial ryegrass cultivars (33 diploids and 30 tetraploids) were sown in experimental plots at 5 locations in 2010 throughout Ireland by the Department of Agriculture, Food and the Marine (DAFM). Site locations were Backweston, Co. Kildare (5° 22′N; 6°30′W); Raphoe, Co. Donegal (54°52′N; 7°36′W); Fermoy, Co. Cork (52°08′N; 8°17′W); Athenry, Co. Galway (53°18′N; 8°45′W) and Kildalton, Co. Kilkenny (52°21′N; 7°20′W). The experiment was a randomized complete block with three replicates of each cultivar at each site. A simulated grazing management (defoliations every 3 – 4 weeks from mid-March to mid-October) was implemented at 4 sites throughout 2011 and 2012. At the Kildalton site, a 2-cut silage

management was imposed, with the first defoliation in early April, followed by the 1st silage harvest in late May, and the 2nd silage harvest in late June. Three aftermath simulated grazings followed, with the final defoliation in mid-October. A total of 310 kg N/ha was applied per year. All plots were harvested to a residual height of approx. 4.5 cm. Harvested material was weighed and a subsample was dried at 80ºC in an oven for 16 hours for DM determination. The dried samples from the Backweston site were milled and analysed for DMD using NIRS technology. Seasonal production was defined as spring (all herbage harvested prior to April 10), mid-season (herbage harvested from April 11 to August 10) and autumn (herbage harvested from August 11 to the final harvest in November). Data from the 1st and 2nd silage harvests within the 2-cut silage management were used to determine 1st and 2nd-cut silage DM yields. Ground score was estimated visually in December 2011 and 2012 at the four sites where the simulated grazing management was imposed. The difference in ground score between the two years was used to determine GSΔ.

The economic values for each trait were determined using the Moorepark Dairy Systems Model (MDSM; Shalloo *et al.*, 2004) which simulated a physical change in each trait of interest independently. The difference between the farm net margin before and after the change was simulated was divided by the change in the trait of interest in order to determine the economic value for a unit change in each trait (McEvoy *et al.*, 2011).

Results and discussion

To investigate the effect of an increase or decrease in cultivar performance within each trait, base values were necessary to predict the economic merit of each cultivar for each trait. The base level of DM production was calculated using the average level of DM production on commercial grassland farms in Ireland (9.1 t DM/ha; Shalloo, 2009). The base values 1.22 (spring), 6.02 (mid-season) and 1.86 t DM/ha (autumn), respectively. The associated economic values are: ±€0.16, ±€0.04 and ±€0.11/ha per year, for each 1 kg difference from the base value for spring, summer, and autumn DM yield, respectively. There are no quality data available on commercial swards; therefore, to determine the base level for quality the VCU dataset of 63 cultivars was used. The base value for quality (g/kg DMD) for each month was as follows: April (849.6), May (848.6), June (814.4) and July (810.2); the associated economic value is ±€0.001, ±€0.008, ±€0.010 and ±€0.009 per unit change in DMD on a g/kg DM basis, for April, May, June and July, respectively. Currently there is no silage DM yield data available on commercial swards. To determine the base level for 1st and 2nd cut silage DM yield the VCU dataset was used. The base value for 1st and 2nd cut silage DM yield was determined using the two years data on 63 cultivars at the Kildalton site, with all the cultivars and all replicates included in the generated values. The base value for 1st and 2nd cut silage DM yield was 3785 and 3692 kg DM/ha, respectively. The associated economic value was ±€0.041 and ±€0.028, for each 1 kg change in silage, relative to the base values, respectively. Current recommendations are to reseed at least 10% of the grassland area each year (Shalloo *et al.*, 2011), with swards expected to last 10 years before regeneration. This indicates the standard sward should last for 10 years at farm-level, and this was the base level for persistency in the model. The total cost of reseeding (€672/ha; M. O'Donovan, unpublished data) divided across the expected 10-year sward persistency, equates to a depreciation cost of €67/ ha per year, but if a sward persists for a shorter period the cost of depreciation increases. Cultivars surviving 10 years or more had no negative value for the persistency trait.

The economic values and the total economic merit for a subset of the 63 cultivars are presented in Table 1. Cultivar 1 had the highest PPI value (€217/ha per year); from the sub-indices it is clear that this cultivar had a high performance in all traits except 1st-cut silage (-€4). When selecting cultivars it is important that a farmer examines the sub-indices to identify which cultivar performs well in the traits required, e.g. if selecting a cultivar for a silage paddock, the

emphasis on 1st and 2nd-cut silage should be increased. Alternatively, if formulating a mixture, cultivars should be selected which complement each other's strengths and weaknesses. The updated economic values reported in this paper have not resulted in any significant change in the rank order of the cultivars outlined below.

Table 1. Total Economic merit of 10 cultivars and their performance in the sub-indices

Cultivar Details			PPI Details	PPI Sub Indexes (€/ha per year)						
			PPI Total €/ha per year	DM Yield			Silage		Quality	Longevity
Cultivar	Ploidy	HD		Spring	Summer	Autumn	1st cut	2nd cut		
1	T	28-May	217	60	51	54	-4	10	47	0
2	D	29-May	188	67	56	89	-2	-15	23	-29
3	T	05-Jun	166	34	57	62	-5	4	31	-17
4	T	20-May	165	76	41	35	17	-4	7	-7
5	T	23-May	147	39	55	22	31	-4	2	0
6	T	5-Jun	137	46	51	26	-18	7	26	0
7	T	01-Jun	129	38	52	21	7	1	10	0
8	T	06-Jun	99	4	58	22	-8	12	29	-17
9	T	04-Jun	94	14	47	6	3	0	24	0
10	D	23-May	67	61	40	28	16	-12	-20	-45

Conclusions

The use of the pasture profit index enables the identification of cultivars that will provide the greatest economic contribution to a ruminant grazing system. The total economic merit clearly identifies the strengths and weaknesses of individual cultivars and will heighten the end-use and commercial sales of superior performing cultivars. More importantly it will allow farmers to choose cultivars that are suitable for the individual requirements of their farm.

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Theme 6 posters

The effect of resistance to mildew infection on ruminal fermentation of *Lolium perenne*

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Abstract

In the field, resistance to microbial pathogens increases crop yield. The work described here was designed to investigate whether the priming of endogenous anti-microbial defence responses associated with resistance mechanisms could affect subsequent utilization by the rumen micro-organisms during colonization and fermentation of the ingested fresh forage. The non-discriminatory approach Fourier Transform Infra-Red Spectroscopy (FTIR) was used to profile the entire metabolome generated during *in vitro* fermentation of *Lolium perenne* leaves which had been previously infected with an avirulent mildew, thus generating localized lesions of plant-cell death as part of the Hypersensitive Response defence reaction. Regardless of mildew exposure there was an effect of incubation time on metabolic profile, with separation becoming more obvious with increasing fermentation time. Within these individual timepoints, inoculation with mildew was also seen to affect the metabolic profile with the profiles of uninfected (control) leaves clustering away from infected leaves at later timepoints. We therefore propose that pre-exposure of grass to infection by avirulent mildew elicits plant defence responses that modify subsequent colonization by the rumen microbial population. The consequences of this for fermentation efficiency are currently being assessed.

Keywords: *Lolium perenne*, pathogen resistance, forage quality

Introduction

In the field, plants are subject to exposure to microbial pathogens. Resistance to pathogens such as mildew involves a cell death response known as the Hypersensitive Response (HR). This forms a localized barrier of dead cells in the region of the developing spore preventing access of the spore to nutrients and hence preventing fungal development (Mur *et al*., 2008). We have previously demonstrated that plant tissues induce stress-related processes associated with cell death and proteolysis on exposure to rumen-like conditions (Kingston-Smith *et al*., 2013; 2012). These defence responses include alterations in metabolism which could affect quality of the ingested feed and provision of nutrients to the colonizing microbial population (Huws *et al*., 2013). Hence we have tested the hypothesis that the process and products of microbial fermentation in the rumen are affected by prior exposure of the fresh forage feed to biotic factors. We have assessed the effect of prior exposure of *Lolium perenne* to four doses of an avirulent strain of mildew (thus inducing HR) on the metabolic profile generated during fermentation to determine the implications of breeding for resistance on post-ingestion metabolism of fresh forage feeds.

Materials and methods

Perennial ryegrass (*Lolium perenne*, cv. AberDart) was grown for six weeks in compost. Six independent replicates were each inoculated with avirulent mildew (previously grown on oat) at approximately 0, 20, 50 and 100 conidia/ mm^2 by use of a settling tower, after which the plants were placed in a growth cabinet for 48 h to allow the infection to develop. For *in vitro* fermentation, Hungate tubes were prepared to contain 0.5 g FW of grass (which had been previously cut to ~ 1 cm lengths) to which a 10% rumen fluid inoculum was added under a CO_2 stream before the tubes were sealed with butyl rubber stoppers. Tubes were placed at 39ºC in

the dark until they were destructively sampled at 0, 2, 4, 6 and 24 h after inoculation. The remainder was fractionated into 'residue' (plant material plus attached, colonizing bacteria), 'pellet' (planktonic phase bacteria) and 'footprint (cell-free liquid phase) samples were for analysis by FTIR as described previously (Kingston-Smith *et al.*, 2013). Principle component analysis of FTIR and metabolite data was performed with Pychem software (Jarvis *et al.*, 2006).

Results and discussion

The main factor affecting the metabolic profile of the plant-microbial interactome formed during fermentation of the grass was time (Figure 1). This was consistent with previous FTIR-based analyses of fresh forage fermentation (Kingston-Smith *et al.*, 2013). Discriminant function analysis (DFA) showed clear separation of 24 h samples away from all others, with evidence of clustering of 6 and 4 h samples. The samples from 0 and 2 h time points were more similar in composition. An effect of treatment on residue composition was also detected (Figure 1). Within each time cluster, uninfected grass (treatment A) separated from those samples which had been previously infected with mildew (treatments B, C & D), for which some evidence for a dose-dependent effect could be detected, especially in samples taken 6 and 24 h after inoculation with rumen fluid. Loadings plot revealed that the sources of these differences were in the regions of the spectra associated primarily with amide bond containing metabolites (Nakanishi, 1963).
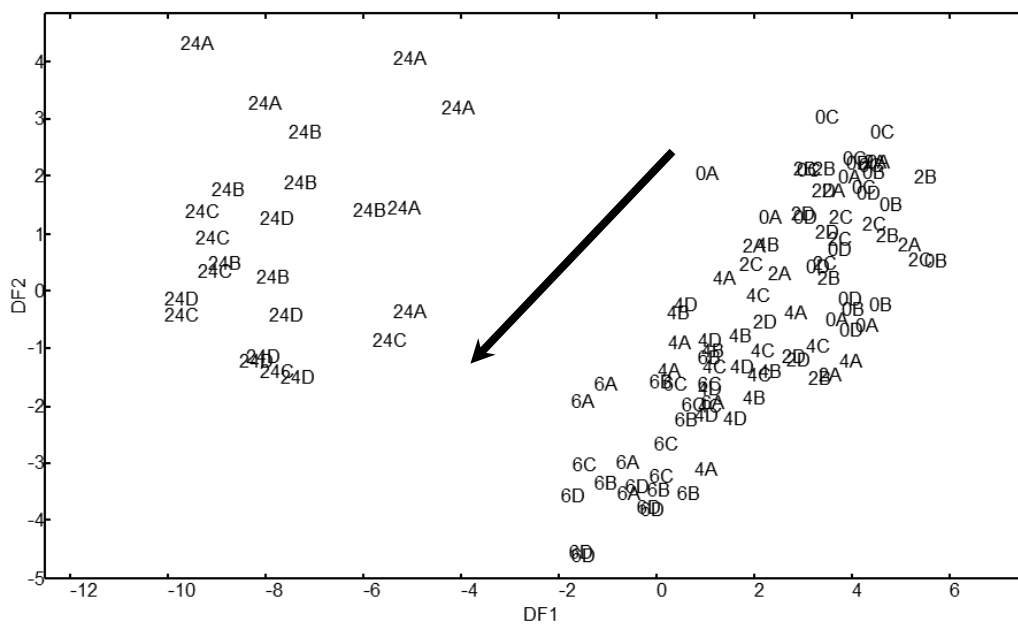
Figure 1. Discriminant Function Analysis (DFA) of FTIR spectra derived from residue samples collected after 0, 2, 4, and 24 h of fermentation of *L. perenne* previously inoculated with avirulent mildew at approximately 0 (A), 20 (B), 50 (C) and 100 (D) conidia/ mm^2. Arrow indicates direction of separation according to treatment. The DFA model is based on 6 principal components representing 99.6% of the total variation of the dataset.
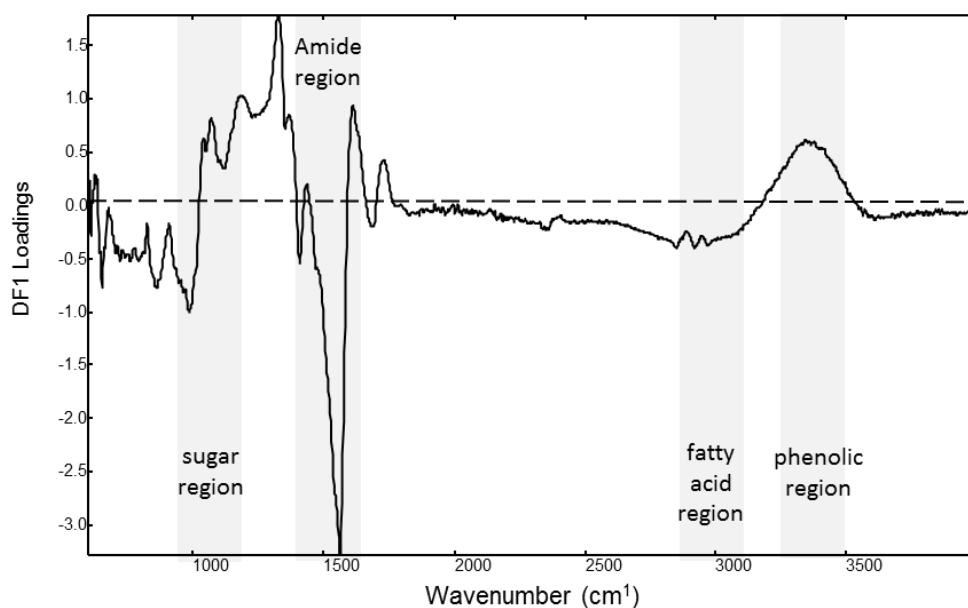
Figure 2. Loadings plot showing the main sources of variation in the FTIR spectra derived from residue samples collected after 0, 2, 4, 6, and 24 h of fermentation of *L. perenne* previously inoculated with avirulent mildew at approximately 0 (A), 20 (B), 50 (C) and 100 (D) conidia/ mm^2. Regions associated with particular molecular groups are shaded in grey. The dotted line indicates a position where no contribution is made to the variation seen in Figure 1.

Conclusion

Pre-infection of forage grass with mildew was shown to affect the metabolic profile generated during fermentation of the forage feed by rumen micro-organisms. These differences could be a consequence of differential nutrient provision because of differences in the metabolome arising as a result of implementation of defence reactions. Alternatively, the pre-exposure of plant cells to a microbial pathogen, and induction of successful resistance mechanisms could cause systemic changes in the forage which alter niche provision during colonization and can perturb the functionality. Ongoing work will determine if these metabolic changes were associated with differential colonisation profiles and the implications of any changes on functionality and rumen efficiency.

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Disease resistance in red clover (*Trifolium pratense* L.) to stem nematodes and *Sclerotinia*

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Abstract

Two important pathogens of red clover in temperate areas are stem nematodes and the fungal disease *Sclerotinia*. The red clover breeding programme at IBERS, Aberystwyth is focusing on reducing the damage to plant persistence caused by these pathogens.

Keywords: *Trifolium pratense*, *Sclerotinia*, stem nematode, DNA fingerprinting

Introduction

Red clover (*Trifolium pratense* L) is an increasingly important forage legume for sustainable grassland systems, producing high dry matter yields of quality forage. However, varieties of this species tend to lack persistence, particularly under grazing. New varieties, such as AberClaret and AberChianti, have demonstrated increased persistence (Marshall *et al*., 2014). In red clover, pests and diseases are major contributors to loss of persistency, in particular crown rot (*Sclerotinia trifoliorum*) and stem nematode (*Ditylenchus dipsaci*). *Sclerotinia trifoliorum* is a difficult fungal infection to control. When soil-borne it is easily transferred and very difficult to eradicate. Once the infection is in the field its presence can mean a long period of non-productivity for the farmer. *Sclerotinia* was one of the reasons for the decreasing use of red clover in the UK during the 1970s and 1980s. Coupled with a potentially high mutation rate when compared to stem nematode, it has potential for recurrent outbreaks in the UK, particularly as wetter, milder winters and drier springs increase in frequency. Stem nematode also has significant effects on red clover yield and persistence, and, although slightly slower to develop, symptoms include severe stunting and death of plants. A major breeding target, and the aim of this project, was to use AberClaret and AberChianti, as well as other elite material and natural populations, in a selection and crossing programme in order to generate red clover populations with improved resistance to these two pathogens. This has now progressed to a stage where multiple generations of material are available for back crossing within both populations. The use of a limited number of molecular markers has enabled us to follow the pedigree, and avoid issues with inbreeding and the use of agronomically inferior material. There is also potential to combine both populations at the F2 generation to create a potential third population resistant to both diseases.

Methods and results

The plant varieties and lines used were: Redhead (nematode-susceptible control), Milvus, Merviot, Formica, AberRuby, Aa4512, Aa4494 (now AberChianti) and Aa4495 (now AberClaret). Each group was duplicated and 120 plants of each variety were tested for both diseases. All plants were inoculated at approximately 8 weeks after germination by inserting the inoculum (liquid for stem nematode and an agar plug for *Sclerotinia*) into the axil containing the developing meristem. A period of high humidity was applied to secure the inoculum into the plant, followed by assessment over a period of time. The assessment period for *Sclerotinia* from the point of infection was six days, with infection scoring recorded on the second, fourth and sixth days. Each plant was then separated into individual pots and further assessed over several months for latent infection. Plants were scored on a 5-point system (0= uninfected; healthy 5= infected, dead). Stem nematode assessment was carried out over a six-to- eight-week

period, depending on the nematode inoculation population. Three scores were recorded at each assessment for the lamina, petiole and meristem at two weeks, four weeks and six weeks.

All of the survivors from both tests were sampled for DNA fingerprinting with microsatellite markers. This allows us to track each plant's pedigree as the project progressed. Plants were then poly-crossed within their appropriate disease groups, harvested and weighed. Initial selection for F1 generation testing included any plant setting seed. Measurements of average seed weight and seed weight distribution were used to select three seed groups within each variety (Low, Average and High seed yield), and selected varieties were planted in field trials to assess agronomic characteristics. The stem nematode programme is currently undergoing F2 screening. Using flower-head weights, seed weights and DNA fingerprinting we have been able to maintain genetic diversity from the original survivor plants. However, the original F1 *Sclerotinia* screen only provided 12 survivors, thus severely reducing the gene pool. Stem nematode resistance is more easily assessed as resistant or susceptible, whereas the *Sclerotinia* screen produces degrees of resistance and tolerance. Resistant material shows no infection, or a limited halo effect around the point of inoculation. Tolerant material can show some initial infection along the petiole, but most importantly not into the meristem. This allows the plant to survive and grow away from the infection, due to HSR (Hyper Sensitive Response). In the first screens, both resistant and tolerant plants were used within the poly-cross. We have now carried out a new disease screen using ecotype and landrace material held within our gene-bank with a reduced number of the original varieties together with new varieties added to maintain genetic diversity. In addition, we now only select resistant plants and leave these plants as long as possible to assess latent infection. As a result we now have 71 resistant plants from three screens, involving a total of over 2700 plants. The resistant material is now being poly-crossed to generate F1 seed.

Discussion

This work forms the basis of a breeding programme providing a wide range of resistant plant material which can be retested and introgressed into other elite material when required. DNA fingerprinting has allowed us to follow the progression of an individual plant's heritage. The ability to trace both maternal and paternal lines of each plant has enabled us to maintain a broad genetic pool of material. The stem nematode programme has advanced furthest, and the marker information has already helped make breeding selection choices within the F2 population selected for screening. This will also be invaluable for the *Sclerotinia* population selections in the future. As *Sclerotinia* evolves, new resistant material can more easily be selected. Stem nematodes are naturally occurring within soil, and it is only when a population increases on a suitable susceptible host that problems occur. This may in part explain why we find a higher resistance rate compared to *Sclerotinia* (71 of 2940) (Table 1).

Table 1. Number of tested and resistant plants in F0 and F1 populations of stem nematode. Control plants have been omitted.

	Number of plants	
	Tested	Resistant
F0	1080	170
F1	850	307

We intend to continue screening through to at least the F4 generation for each disease group and start a programme of back crossing and screening into elite lines with another field trial at the F3 stage. We also plan to combine both F2 generations in one poly-cross in order to attempt to identify resistant plants to both *Sclerotinia* and stem nematode.

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Selection of white clover (*Trifolium repens* L.) for improved phosphorus use efficiency

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Abstract

The development of high yielding white clover (*Trifolium repens* L.) varieties with improved phosphorus use efficiency has potential environmental benefits as well as economic benefits to the grassland sector, challenged as it is to reduce the environmental consequences of production through sustainable intensification. Survivor plants were collected from a long term, low input trial and crossed to identify germplasm with enhanced phosphorus use efficiency (PUE). Several lines were identified that showed improved performance relative to control varieties under P-limitation. This also related to the performance of the companion ryegrass.

Keywords: *Trifolium repens*, PUE, variety development

Introduction

Phosphorus (P) is an essential macronutrient of plants. It serves as a structural element in nucleic acids and phospholipids, and plays a central role in biochemical energy transfer. External application of phosphorus fertilizer, either through the use of mineral phosphate or livestock manures, has a marked positive effect on agricultural crop productivity. Cultivated legume species have long assumed to have a particularly high demand for P for nodulation (Marschner, 1997), however, the veracity of this has been challenged (Sprent, 1999).

Most phosphorus destined for use in agriculture is derived from phosphate rock and extracted through mining. The world's major reserves of rock phosphate are in the control of relatively few countries, including Morocco, China and the US. Demand for P is increasing concomitantly with increased global demand for food, yet reserves of rock phosphate are being rapidly depleted and are expected to run out in the next 50 to 100 years (Cordell *et al*., 2009). Moreover, surplus soil phosphate is a significant contributor to the eutrophication of freshwater systems and over-use is discouraged (Lemercier *et al*., 2008). Indeed, the EU water framework directive seeks to address this problem. A demand thus exists for improved varieties of crop plants that are able to perform competitively under low P fertilization regimes, whether through conservation of use or through an enhanced acquisition or uptake (Vance *et al*. 2003, Vance 2011).

Mass selection provides a simple yet effective method for genetic improvement of outbreeding crops (Acquaah, 2012). Selection of individual plants within a population is made on the basis of desirable characteristics or through the elimination of individuals with undesirable characteristics. The selected plants are intercrossed and the expectation is that the resulting progeny, assuming reasonable heritability, will be improved for the characteristics in question relative to the original population. In this study we report on the selection of survivor plants from a long-term, low-input trial as a method for selecting for phosphate use efficiency (PUE).

Materials and Methods

Survivor plants were collected from a long term, low input trial at Bronydd Mawr Upland Research Centre, Brecon, Wales. These comprised four populations of small leaved white clover, each of 40 survivor plants. The populations were individually polycrossed in a glasshouse using insect pollinators and the resulting F1 seed sown. The 50 most vigourous F1 plants from each population were taken and polycrossed again, producing four low P families

(LPF1-4). The F1 generation were also analysed for foliar P content and 50 plants with high foliar P content, taken randomly from the four populations, were polycrossed to give a "high foliar P" family (High FP). Similarly, 50 plants with low foliar P content, taken randomly from the four populations were polycrossed to give a "low foliar P" family (Low FP). These six lines were sown in March 2011, along with the control varieties AberAce and AberAtom (both small leaved white clover varieties), in replicated plots (six of each line or variety) in a field exhibiting low soil P (2.43 ± 0.14 ppm) at IBERS, Gogerddan near Aberystwyth, Ceredigion, Wales. Plot sizes were 5 m x 1.2 m. Sowing rates were 2.1 g of white clover seed and 10.8 g of the companion perennial ryegrass (*Lolium perenne* L.) seed (cv. Premium) per plot. Plots were managed as per National List trial guidelines *i.e.* plots were split in two with half of the plots left unfertilized with P while the other half were fertilized at a rate of 175 kg ha^{-1} P$_2$O$_5$ a^{-1}. The plots were harvested with a Haldrup forage harvester at a cutting height of 5 cm. Harvests were taken three times in 2011, and twice each in 2012 and 2013 (reflecting poor growing conditions). Fresh weights were measured and grass and white 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 18 hours.

Results and discussion

As expected, P treatment had a marked effect on DM yield, both of white clover and total DM yield. The two control varieties were broadly similar, with the untreated plots having 50-54 % of the white clover DM yield and 67-69 % of the total DM yield of the P treated plots. Yields were in line with those expected from plots of this nature, incorporating small leaved white clover and perennial ryegrass varieties. The low P families derived from survivor plants from the long term low input trial (LPF1-4) gave variable results. Of the four families, in terms of white clover DM yield, only LPF2 showed any improvement relative to AberAce, but was indistinguishable from the other control variety AberAtom. The remaining three families were either comparable or lower yielding than AberAce.

Table 1: Mean annual dry matter yields (kg ha^{-1} a^{-1} dm ± se). Columns show yields without phosphate treatment (-P) and with phosphate treatment (+P) as well as the ratio of -P yield to +P yield. Results are presented as means of three harvest years' data.

	Clover yield			Total yield		
	-P	+P	Ratio	-P	+P	Ratio
AberAce	846 ± 109	1568 ± 133	0.54	5754 ± 375	8527 ± 145	0.67
AberAtom	935 ± 83	1860 ± 275	0.50	5641 ± 244	8211 ± 202	0.69
LPF1	540 ± 53	1230 ± 201	0.44	4902 ± 38	8067 ± 125	0.61
LPF2	1154 ± 172	1710 ± 211	0.67	7131 ± 124	9272 ± 20	0.77
LPF3	651 ± 98	1306 ± 187	0.50	7378 ± 204	7019 ± 110	1.05
LPF4	648 ± 178	1172 ± 128	0.55	6111 ± 203	7832 ± 77	0.78
Low FP	668 ± 138	1429 ± 114	0.47	5742 ± 156	7312 ± 43	0.79
High FP	523 ± 134	1336 ± 206	0.39	5097 ± 122	7297 ± 158	0.70

Where there was significant improvement in the LPF families was in total DM yield. Both LPF2 and 3 yielded significantly better than both control varieties in the untreated plots, with LPF2 also out-yielding the control varieties in the P treated plots. LPF2, 3 and 4 also showed a lower overall response to P application than the control varieties, as seen in their higher -P DM yield/+P DM yield ratio. A similarly reduced response was seen in the low foliar P family (Low FP), although there was no measurable increase in total DM yield.

The improvement in total DM yield in LPF2 and more generally the lower response to application of P in the LPF families indicates that PUE is a trait that can be selected for, and that collection of survivor plants from low-input systems is a useful means to identify

germplasm for this trait. The mechanism by which the LPF families perform better in P limited conditions appears to affect the companion grass as well as the white clover, given that much of the improvement in DM yield, particularly in LPF2, relates to grass DM yield. What this might be is currently unknown, but may relate to variation in the ability of white clover to acquire P from the rhizosphere, resulting in improved supply to both species. Alternatively it may be due to enhanced potential for nitrogen fixation in low P conditions.

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Selection of contrasting cold-tolerant white clover genotypes from twenty-eight populations naturalized in southern Chile and Argentina

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Abstract

A collection of 28 populations of naturalized white clover in Argentinean and Chilean Patagonia, and two white clover cultivars, was used to select two groups of contrasting cold tolerance genotypes: 96 cold-sensitive and 96 cold-tolerant. The objective was to form an association-mapping population. Sixty young plants of each population were cold stressed progressively at -2, -4, -6 and -8 ºC for 48 hours and the damaged plants recorded. The records were fitted to the Weibull distribution and the three most tolerant and the three most sensitive populations were selected using the LT50 value (Lethal Temperature for 50% of the population) to choose the contrasting genotypes. The white clover populations showed a broad genetic variability for cold tolerance, which allowed the selection of 192 genotypes with divergent cold-tolerance and formation of the association-mapping populations.

Keywords: Cold tolerance, naturalized populations, Weibull distribution, plant survival

Introduction

An important aspect of the interaction between white clover and grass in a grass-clover mixture is the lower clover growth rate at low temperatures (5-15 °C), which affects the clover competitiveness during early spring and late autumn, resulting in a low contribution of white clover to total yield. White clover is naturalized in Chile from the central regions to the extreme south, covering a broad range of soil types, altitude, climates and latitude (30 to 55° S). The forage species germplasm collection developed during the 1990s with material obtained from Argentinean and Chilean Patagonia (from 37 to 55° S), gathered 28 populations conserved at the INIA forage germplasm bank in Temuco. It is expected that these naturalized populations would have adapted to the specific environmental conditions, including cold environments, from where they were collected, after one or two centuries from their introduction to each particular region (Svenning *et al.*, 1997).

This collection, in addition to two white clover cultivars, was used for selecting the contrasting cold-tolerant genotypes using young plants subjected to progressive low temperatures in a frost chamber. The selection of contrasting germplasm would allow the identification of genotypes with a broad range of molecular and phenotypic patterns necessary for association mapping studies. The objective of this work was to develop an association-mapping population composed of 192 genotypes with divergent cold tolerance (96 sensitive and 96 tolerant) which would subsequently be subjected to molecular and phenotypic characterization under field conditions.

Materials and methods

During the summer of 1994, 1995 and 1996, twenty-eight white clover populations were collected as seeds in the sites described in Table1. The white clover cultivars included were Haifa and Weka. Sixty seeds were taken from each population and germinated in nursery containers with 128 small holes of 27 cm³ (3×3×3 cm) under greenhouse conditions. Sterile peat was used as the substrate. Periodically the 1800 plants (30 populations × 60 seeds) were watered with Hoagland's medium nutrient solution (0.5 X) and one week after emergence were

inoculated with specific rhizobium. Forty days after germination the plants were transplanted into small pots of 216 cm^3 (6×6×6 cm) and grown until the first stolons reached around 10 cm length. During July and August the plants were subjected to progressively lower temperatures (-2, -4, -6, and -8 ºC) in a cold chamber for 48 hours at each temperature. Due to the chamber capacity the plants were treated in three groups of 20 plants of each population. At the conclusion of each cold period the number of damaged plants (all leaves and the thinner stolons dead) was counted. The plant survival records and the environmental temperatures were fitted to a Weibull distribution (WD). The shape and scale parameters were estimated for each population using Statgraphics V15 Software. Also, the LT50 (Lethal Temperature for 50% of the population) was estimated from the survival function of WD. The three populations with the highest LT50 were considered the most tolerant and the three which presented the lowest LT50 were considered the most sensitive to cold. From these two groups of populations the 96 most cold tolerant genotypes and the 96 most cold-sensitive genotypes were selected as follows: all the genotypes without damage at -8 ºC from each population, plus plants damaged at -6ºC chosen at random to complete 32 plants when necessary (tolerant); and all the genotypes damaged at -2, -4 and -6 ºC plus genotypes damaged at -8 ºC chosen at random to complete 32 plants when necessary (sensitive).

Table 1. Geographical information of collection sites of 28 white clover population and two cultivars.

Pop	Georeference (hddd°m")	Altitude (m)	CO	Pop	Georeference (hddd°m")	Altitude (m)	CO	Pop	Georeference (hddd°m")	Alt (m)	CO
94-14	S39 25 W72 11	nr	CL	94-38	S40 07 W71 39	1050	AR	94-62	S39 10 W71 15	990	AR
94-18	S40 00 W72 33	250	CL	94-41	S39 46 W71 37	820	AR	94-65	S39 33 W70 57	120	AR
94-19	S40 16 W72 39	220	CL	94-45	S39 54 W71 36	810	AR	95-20	S44 15 W71 50	nr	CL
94-22	S40 30 W72 14	nr	CL	94-46	S40 00 W71 30	890	AR	95-36	S45 34 W72 06	nr	CL
94-24	S40 23 W72 47	nr	CL	94-50	S39 37 W71 23	880	AR	95-71	S46 15 W71 52	nr	CL
94-28	S40 48 W71 46	820	AR	94-51	S39 37 W71 23	880	AR	95-77	S47 09 W72 21	nr	CL
94-30	S40 40 W71 53	960	AR	94-52	S39 35 W71 27	880	AR	96-33	S51 34 W72 31	nr	CL
94-33	S40 26 W71 36	970	AR	94-54	S39 33 W71 25	890	AR	96-64	S52 43 W71 03	0	CL
94-35	S39 56 W71 38	850	AR	94-55	S39 04 W71 12	1400	AR	Weka			AU
94-36	S39 57 W71 40	850	AR	94-58	S39 19 W71 03	1290	AR	Haifa			OL

*CO: country of origin (CL, AR, AU and IL for Chile, Argentina, Australia and Israel, respectively)

Results and discussion

Controlled cooling has been described as an indirect and highly effective selection method for improving cold tolerance in perennial forage species (Waldron *et al*., 1998; Castonguay *et al*., 2009). In this work, successively cold-stressing young white clover plants at -2, -4, -6 and -8 °C allowed us to observe a high genetic variability for plant/tissue survival between the 30 populations. When the temperature decreased the percentage of damaged plants increased from 1.8% (-2ºC) to 70.0 % (-8ºC) (Figure 1a). The highest variations among populations were observed at -6 and -8 ºC, fluctuating in ranges of 1.7 - 76.7% and 23.3 - 100% of damaged plants, respectively (Figure 1a). The Haifa and Weka cultivars differed broadly in genetic backgrounds due to their origin. Haifa was developed in Israel in a Mediterranean environment, and therefore it shows low winter growth and high spring-summer growth due to its tolerance of thermic stress. On the other hand, Weka was developed in Australia and bred for improving its autumn–winter productivity. In spite of these differences, both cultivars showed similar performances under the conditions imposed in this experiment, where they presented a threshold of damage similar to the most sensitive naturalized populations (Figure 1b). The response of the white clover naturalized populations (survival) to the environmental temperature fall was fitted reasonably to the WD (D test=0.091; *P*>0.05). The shape parameter of the distribution curves, estimated for each population, varied broadly from 4.5 to 31.7. In general, the most cold-sensitive populations showed a low value of the shape parameter, which

means an abrupt decrease in survival when the cold increases (Figure 1b). The LT50 was estimated from the survival function of the WD (Figure 1b). Populations 94-22, 94-36 and 94-54 presented the lowest values of LT50 (-7.2 °C), and populations 94-65, 94-19 and 94-30 showed the highest values of LT-50 (-8.4 °C). The cultivars Haifa and Weka showed the same value of LT-50 as the cold-sensitive populations. The effectiveness of the methodologies applied to white clover in this experiment is being evaluated under field conditions in three environments, with a gradient of cold associated with altitude (100 to 1500 meters above sea level).
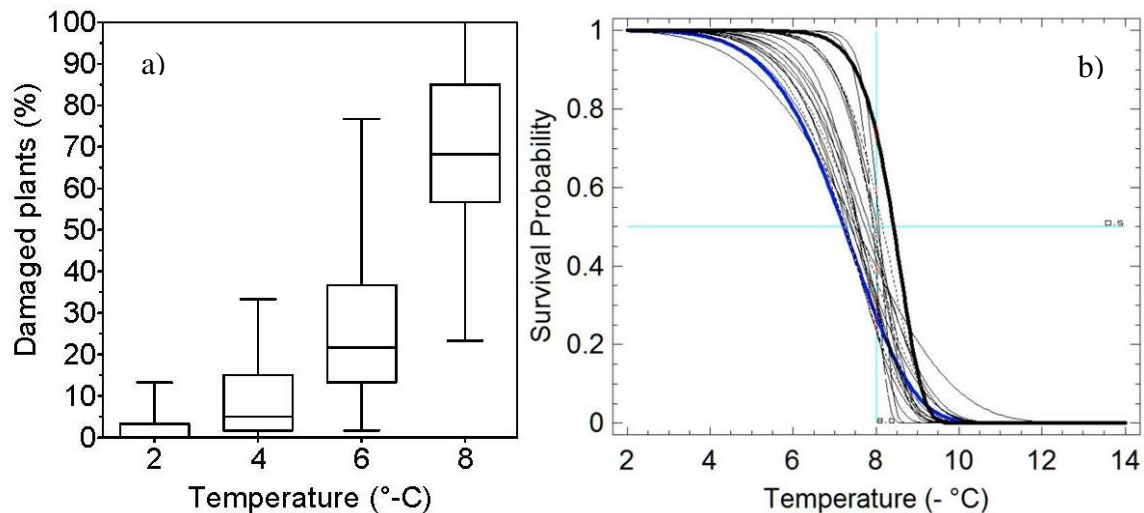
Figure 1. Percentage of damaged plants (a) and plant survival probability curves of 30 white clover populations subjected to a gradient of low temperatures (-2, -4, -6 and -8 °C). Bold lines are the most sensitive and most tolerant populations.

Conclusions

The white clover populations showed a broad genetic variability for cold tolerance, which allowed the selection of 192 genotypes with divergent cold-tolerance and formation of an association-mapping population. The Weibull curve and its parameters may be used as a selection criterion for cold-tolerant genotypes.

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Analysis of changes in population structure over time in components of multi-species swards

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Abstract

In multi-species swards, the genetic structure of populations of heterogeneous outbreeding species is likely to change over time, potentially affecting inter-species population dynamics. In this study, mixtures of outbreeding forage crops were established in swards, and periodically defoliated by cutting or grazing for two years. Changes in population structure varied from species to species. White clover showed significant genetic change over two years, while red clover did not. For perennial ryegrass, a sub group in the initial population exhibiting unique alleles was no longer present two years later. Key genes associated with stress tolerance and flowering time varied significantly with these changes in population structure.

Keywords: population structure, allele frequency, red clover, white clover, perennial ryegrass

Introduction

Programmes of forage germplasm improvement usually result in synthetic varieties derived from intercrossing between small numbers of parental genotypes. However, many of the major components of temperate grasslands are outbreeding, heterozygous perennial species. This means that they contain considerable within-population genetic variation. Consequently, although a successful forage variety released in the EU must satisfy the requirement of distinctiveness, uniformity and stability (as well as value for cultivation and use), it will nevertheless be composed of genetically distinct individuals. Because of this, within-species variation in traits is an important aspect of grassland diversity. Given the high degree of genetic variation present in outbreeding forages, genetic change will inevitably occur in pastures over time, and this is likely to affect species interactions and dynamics. In sown pastures it is well known that the establishment year is characterized by high genotype mortality rates and a decrease in variability among the survivors (Charles, 1961).

A Common Experiment (CE) was set up within the EU-FP7 project 'Multisward' across a subset of partner sites to analyse responses of multispecies swards (MSS), compared with highly fertilized perennial ryegrass (PRG) monocultures, to contrasting managements. The CE imposed grazing and cutting managements typical of intensive production systems for 2-3 years on sward types differing in species number and composition. Within each sward type, grazed and cut plots received the same external applications of nitrogen fertilizer (N) and were defoliated at the same frequency to the same residual height. In the Aberystwyth site we established CE plots containing mixtures of four species (two legumes and two grasses) and analysed the degree of genetic change occurring in populations over time. We compared allele frequencies in the starting population (i.e. before the defoliation managements were imposed), and populations sampled in plots exposed to six harvests per year for two years under cutting and grazing managements.

The markers employed for genotyping comprised simple sequence repeats (SSR) that had previously shown good allelic variation, and single nucleotide polymorphisms (SNP) and SSR in known genes. Some of these genes are involved in the expression or biosynthesis of abscisic acid (ABA) and jasmonate, two plant hormones linked to stress tolerance and response. ABA plays important roles in environmental stress responses (Liming and Jian-Kang, 2003). Jasmonate is crucial to wounding response signalling: deficiency negatively affects a plant's

tolerance of herbivore damage such as grazing (Howe and Jander, 2008). Other marker genes are linked to salt stress, desiccation or long day flowering time (Skøt *et al.,* 2011) Any allelic differences could assist further characterization of these genes, as well as our understanding of the genetic basis of any changes in populations.

Materials and methods

Four forage species in common agronomic use in Europe were included in the CE in Aberystwyth: two grasses (perennial ryegrass - PRG; tall fescue – FA) and two legumes (white clover – WC; red clover – RC). Plots were sown in September 2010. The effect of defoliation management on genetic change in MSS was analysed over time in three of these species (PRG, WC and RC) in both cut and grazed plots of the four-species mixture. Leaf samples were collected from 45 genotypes of each species at 'time zero' (before defoliation treatments were imposed in summer 2011), and at the end of the experiment in October 2013.

The WC populations were genetically screened using 345 AFLP markers. Samples of RC and PRG were each analysed using 15 SSR markers. Additionally, the samples were screened for predicted SNP and SSR in known genes (Table 1). The resulting data were analysed for allele frequency and population structure.

Results and discussion

The population structure results varied between species. A principal component (PCoA) analysis of the WC AFLP data showed that the populations from the two years were genetically distinct (Fig 1a). Analysis of molecular variance (AMOVA) showed that 97% could be attributed to within-population, and only 3% to among-population variation. Interestingly, four markers were identified as being outliers in terms of population structure differentiation, so are possibly located in a genomic region under selection.
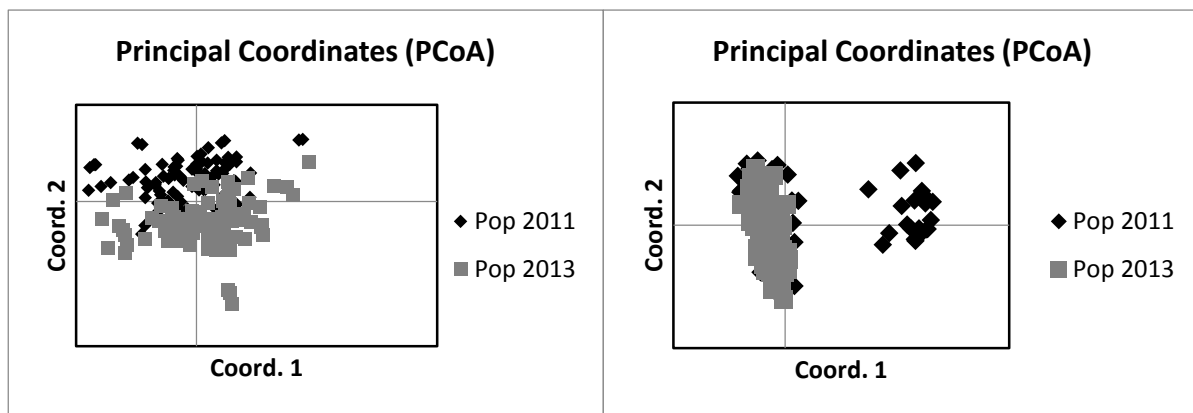
Figure 1a: PCoA of AFLP data for white clover (WC), comparing the 2011 population with 2013.

Figure 1b: PCoA analysis of SSR data for perennial ryegrass (PRG), comparing the 2011 population with 2013.

PCoA and population structure analysis of the 2011 PRG population showed the existence of a small but genetically distinct sub-population. This sub-population was not identified in 2013 (Figure 1b). Some of the genes in the 2011 sub-population had unique alleles. This was particularly notable for FT-LD1, a gene associated with flowering time (Skøt *et al*., 2011). The 2011 sub-population contained two alleles not present in the IBERS PRG breeding population. Their absence in the 2013 population could be due to selection pressure or genetic drift.

RC showed a large number of SNP markers and good allelic variation between individuals. However, there was no significant difference in population or allele frequencies between the 2011 and 2013 populations. Population structure analysis suggested that no admixture had

occurred within the population. This suggests that the RC population remained relatively unchanged during the two-year period with few or no new additional plants from new seed.

Table 1. Key genes showing SNP and SSR variation in red clover (RC) and perennial ryegrass (PRG).

Species	Gene	Putative / known functions
RC	NCED	Abscisic acid biosynthesis (environmental stress response)
PRG	SAG29	Senescence-associated salt-stress protein (Abscisic acid dependant pathway)
RC & PRG	Aquaporin	Water absorption (link with salt stress)
PRG	SRS1	Salt-stress root protein
PRG	Jasmonate Induced Protein	Defence, wounding-induced (relevance to grazing)
RC & PRG	Allene Oxide Synthase	Catalyses biosynthesis of jasmonate
PRG	ABC1	Oxidative stress-related ABC1-like protein
RC	PCC13-62	Response to desiccation
RC	Nitrate Reductase	Reduces nitrate to nitrite
RC	P5CS	Proline biosynthesis
RC	Epimerase Dehydratase	Sugar nucleotide metabolic process
RC	TpZIP	Fatty Acid Desaturase (chlorophyll biosynthesis)
PRG	FT_LD1	Long day flowering time
PRG	PRO1 (isotig06929)	Prostrate growth to erect growth patterns

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Temporal genetic shifts in mono- and bi-specific swards of perennial ryegrass and red clover

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Abstract

Understanding the temporal changes in species composition and within-species genetic diversity in mixed swards is important for the development of field management approaches that exploit the whole potential of the combined species. Here we present the changes in SSR-locus diversity over time in populations of perennial ryegrass and red clover. Standard commercial varieties of both species were sown either in monoculture or in bi-specific swards. Field plots were managed according to standard practice, with 3-5 cuts per year. SSR markers were used to determine the genetic composition of the original and the survivor populations. Genetic shifts over a period of three years were generally small. Over time, perennial ryegrass populations became differentiated from each other in a predictable way. The behaviour of red clover populations was more unpredictable, indicating that local and/or random selection effects played a more relevant role for this species.

Keywords: *Trifolium pratense*, *Lolium perenne*, yield, persistence, genetic shifts

Introduction

The production of forage 'on farm' is an important aspect of sustainable livestock production. Red clover (*Trifolium pratense* L.) and perennial ryegrass (*Lolium perenne* L.) are two of the most used forage species in temperate regions. In Flanders, mixed swards of these crops can render annual dry matter yields of 13.0-18.9 ton/ha (De Vliegher, 2007) of forage with excellent nutritive properties in terms of crude protein. One of the main uncertainties associated with these bi-specific swards is the persistence of the individual species over time. In particular, red clover displays low persistence (Boller *et al.*, 2010). Although perennial ryegrass is more persistent than red clover, probably only a subset of the genotypes will persist over several growth seasons, while others will disappear. The dynamics of both species in mixed swards depends on the properties of the varieties chosen and inter-specific interactions, but environmental and random effects also play a role in the determination of the genetic composition at a given moment in time. This can have an influence on the sward yield and its spread over the growth season. We have investigated the temporal genetic shifts experienced by bi-specific swards of these two species over a period of 3 years, and compared it to the dynamics of monocultures. Commercial varieties with contrasting properties expected to affect the performance of the plants in the sward were chosen for analysis.

Materials and methods

The perennial ryegrass varieties Merks (high tillering) and Meloni (low tillering), and the red clover varieties Crossway (creeping, highly branched) and Lemmon (erect, medium branched) (Cnops *et al.*, 2010; Saracutu *et al.*, 2010; VanMinnebruggen *et al.*, 2014) were used (Table 1). Field plots (6 m x 1.4 m) were established in April 2011 at ILVO, Merelbeke (51° 00' N 3° 48' E, on sandy loam soil) in two replications (A and B). The plots were harvested three (2011), four (2012) or five (2013) times during the following seasons with a Haldrup forage harvester at a height of 6 cm. Ryegrass and clover weight proportions were determined in a subsample of 300-500 g. Dry matter yields were calculated after drying in a ventilated oven (Vötsch) at 75 °C for 48 hours. In the spring of 2011 (T1) and 2013 (T5) leaf samples were harvested for

SSR-analysis. In monocultures of red clover (M04), 40 leaf samples were taken at each sampling moment, in monoculture ryegrass (M01) 60 samples, and in the mixtures (M06 and M07) 40 ryegrass and 30 clover samples were taken. These proportions were determined according to the different number of seeds of each species used for the establishment of the plots (Table 1).

Table 1. Initial composition of the plots. Mono- and bi-specific plots were sown according to agricultural practice (70% grass: 30% clover). Figures represent the percentage of each species at sowing. The estimated number of viable seeds per m^2 is given in brackets.

Plot	*Lolium perenne*		*Trifolium pratense*	
	Merks	Meloni	Crossway	Lemmon
M01	1.0 (1400)			
M04				1.0 (700)
M06	0.35 (490)	0.35 (490)		0.3 (210)
M07	0.7 (980)		0.15 (105)	0.15 (105)

SSR analysis in perennial ryegrass was carried out using a set of 12 primer pairs, amplified in two multiplex sets. For SSR analysis in red clover we used a set of 18 primer pairs, amplified in two multiplexes. FSTAT 2.9.3.2 was used to estimate the alleleic richeness (Ar) and pair-wise F_{ST} values; observed heterozygosities (1-Qintra) were calculated in GENEPOP 4.2; Pair-wise F_{ST} values were used to construct a UPGMA tree in STATISTICA 11.

Results and discussion

Immediately after establishment (T1) most bi-specific plots were dominated by *L. perenne*, with the exception of M06A, in which serious weed contamination occurred (e.g. weeds represented 40.2% of the dry weight at T1 in M06A). Three years later (T5) the opposite relation was observed, with all plots dominated by *T. pratense*. This was confirmed when all the cuts of 2013 were combined (results not shown), indicating a high persistence of the red clover variety(-ies) used in this experiment.

Table 2. Genetic diversity at two sampling times. 'Ar': allelic richness, '1-Qintra': observed heterozygosity. '%Lp' and '%Tp': dry weight contribution of each species to the spring cut.

Time	Plot	*Lolium perenne*			*Trifolium pratense*		
		Ar	1-Qintra	% Lp	Ar	1-Qintra	%Tp
T1	M01A	4.78	0.79				
	M01B	4.78	0.72				
	M04A				7.04	0.63	
	M04B				6.85	0.35	
	M06A	5.75	0.75	14.1	7.04	0.43	45.7
	M06B	5.50	0.65	66.8	6.66	0.47	25.3
	M07A	4.57	0.81	61.9	7.39	0.51	21.6
	M07B	4.88	0.76	58.0	7.83	0.61	28.7
T5	M01A	4.93	0.78				
	M01B	5.12	0.70				
	M04A				7.48	0.60	
	M04B				7.15	0.54	
	M06A	5.80	0.44	47.2	7.13	0.50	52.8
	M06B	5.18	0.63	39.3	7.28	0.55	60.7
	M07A	4.54	0.77	42.1	7.03	0.54	57.9
	M07B	4.81	0.67	26.8	6.90	0.48	73.2

In general terms, the allelic richness changed little over time (Table 2), as confirmed by non-significant t-test results obtained in comparisons of allelic richness at T1 and at T5 (*L. perenne* P=0.94; *T. pratense* P=0.89). In both species, the highest allelic richness at T1 was found in the plots in which two varieties were combined (M06 for *L. perenne* and M07 for *T. pratense*),

but the differences were not large. At T5 this was still the case only for ryegrass. In both species, t-tests demonstrated that heterozygosity levels were similar at both sampling moments (*L. perenne P*=0.14; *T. pratense P*=0.47), confirming that no large reductions in diversity had taken place over three years. Remarkable exceptions were *L. perenne* in M06A and *T. pratense* in M07B, in both cases plots in which two varieties of the corresponding species were combined. It might indicate selective loss of one of the varieties over time. This is probably not the most plausible explanation for ryegrass as, if this was the case, a differentiation between the M06A and M06B plots at T5 would be expected, but this was not observed (see Figure 1A and further in the text). In contrast, for red clover the M07A and M07B plots became clearly differentiated at T5 (see Figure 1B and further in the text).

In general, pair-wise F_{ST} values were low and only in some cases significant (results not shown). To investigate the general patterns of genetic differentiation among plots and sampling moments, UPGMA trees were derived from the F_{ST} values (Figure 1). In perennial ryegrass, T1 and T5 were clearly differentiated, and plots with only Merks (M01 and M07) clustered separate from plots in which Merks and Meloni were combined (M06). The pattern was more complicated in red clover. In this case, the M07 plots at T1 (Crossway and Lemmon combined) were clearly different from others. At T5 M07A had become genetically similar to plots in with only Lemmon (M04 and M06), which could indicate that Crossway might have disappeared over time. Comparison of A and B in Figure 1 suggests a larger influence of random factors for red clover than for perennial ryegrass, with larger divergence over time between A and B plots of the same treatment in red clover.
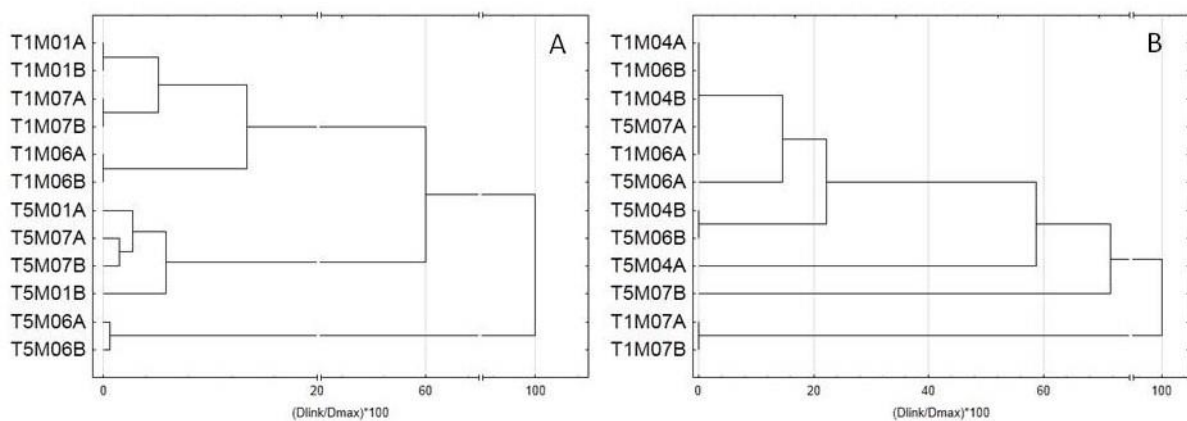
Figure 1. Genetic relationships at two sampling moments (T1 and T5), based on pair-wise F_{ST} values. A) *L. perenne*; B) *T. pratense*. Note: be aware of the breakpoints in the X-axes.

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Persistence of red clover (*Trifolium pratense* L.) varieties in mixed swards over four harvest years

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Abstract

Improving persistence of red clover (*Trifolium pratense* L.) varieties is an important target of the IBERS red clover breeding programme. Identification of the factors contributing to poor persistence and testing improved varieties in field trials are integral to the breeding of persistent red clover varieties. Red clover varieties and selection lines bred for greater persistence were grown in mixed swards with hybrid ryegrass or a mixture of hybrid ryegrass and perennial ryegrass over four harvest years. A significant difference in the DM yield of the red clover varieties was observed, with yield in harvest year 1 greater than in harvest year 4. The red clover varieties differed in the extent of this decline, due to differences in the persistence of red clover plants within swards. The implication of these results for the use of red clover in sustainable grassland systems is discussed.

Keywords: *Trifolium pratense*, yield, persistence, variety development

Introduction

Sustainable livestock production systems are increasingly reliant on the production of high quality forage that can be produced 'on farm'. The high protein content of red clover (*Trifolium pratense* L.) makes it an increasingly important forage legume in such systems producing high dry matter yields of good quality forage (Frame *et al*., 1997). Despite the merits of red clover, under a typical UK management of 3 conservation cuts per year and a late autumn grazing, red clover-based swards tend to persist for only 2 to 3 years after which dry matter yields decline. Red clover varieties that are high yielding for up to 4 harvest years would be advantageous. Identification of the factors contributing to the poor persistence of red clover within swards and applying this information to the development of red clover varieties that combine high forage yields with greater persistence is the primary aim of the IBERS red clover breeding programme (Marshall *et al*., 2012). In spaced plants crown diameter is the morphological characteristic most associated with plant mortality and this was used as a selection criteria to develop red clover varieties that were more persistent. A previous paper described the dry matter (DM) yield of these red clover varieties over three harvest years (Marshall *et al*., 2012). This paper includes results from a subsequent harvest year quantifying the yield of these new varieties and selection lines in comparison with current commercially available varieties.

Materials and methods

Field plots (5m × 1m) 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 in mixed plots sown with hybrid ryegrass (*Lolium boucheanum* Kunth) cv. AberEcho sown at 35kg/ha or with a mixture of perennial ryegrass (*Lolium perenne* L.) cv. AberDart sown at 12.6 kg/ha and hybrid ryegrass cv. AberEcho sown at 22.4 kg/ha. All plots were harvested three times in each of the following four 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. In spring of harvest year four two 0.25 m^2 quadrats were placed at random in each plot

and the number of red clover plants within each quadrat recorded. The experiment was a split plot design with three replicate blocks with grass mixture as main plots and varieties as sub-plots.

Results and discussion

Red clover has an 18-19% crude protein content (Frame *et al.*, 1997) and can be grown across the UK. However, it lacks persistence in mixed swards and yields tend to decline after the second harvest year. Increasing the persistence and yield of red clover is increasingly recognized as essential to capitalize on its high forage quality. Previous studies have shown differences in the DM yield of red clover varieties over three harvest years (Marshall *et al.*, 2012) but also highlighted the decline in yield of some varieties after the second harvest year. Yields of all the varieties and selection lines within the experiment declined in the fourth harvest year compared with year 1 (Figure 1). Total yields were generally similar between years 1 and 4; however, the greatest difference was in the red clover DM yield (Figure 1), leading to a lower proportion of red clover in the swards. The red clover DM yield of the varieties ranged from 7.5 to 22.6 t ha^{-1} in year 1 and from 1.54 to 10.2 t ha^{-1} in year 4, while the highest total DM yield in each harvest year was 27.4 t ha^{-1} in year 1 and 21.01 t ha^{-1} in year 4 (Table 1). The varieties AberClaret, AberChianti and the selection line Aa4559 have been selected for improved persistence and yield. Although the yield of these varieties also declined, the difference in yield between years 1 and 4 was relatively small compared to the control varieties Milvus and Merviot.
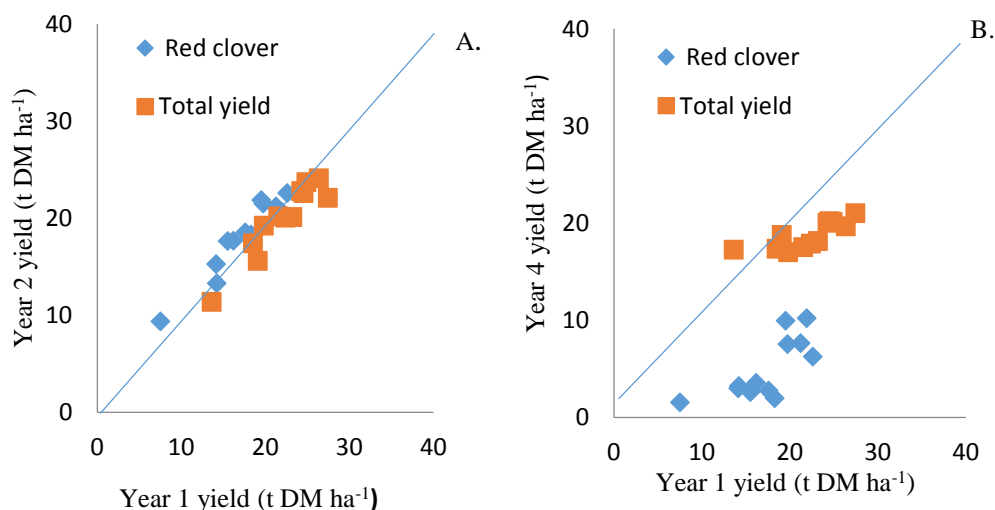
Figure 1. Red clover and total (red clover + grass) dry matter yield (t ha^{-1}) of 12 red clover varieties and selection lines grown in mixed swards. A) Yields in year 1 and year 2; B) Yields in year 1 and year 4.

The decline in DM yield is a consequence of a loss in red clover plants per unit area, which is greater in some of the less-persistent varieties (Table 1). Plant density in spring of year 4 was highest in mixtures containing AberChianti and Aa4559 (23.3), and lowest in mixtures containing Merviot (6.0). The plant density results also support the observation that field trials of three-years duration are insufficient to fully show differences in persistence between red clover varieties - increasing the duration of field trials beyond three harvest years is essential to provide good evidence of differences in persistence between varieties. This also has implications for official variety testing systems. The current UK red clover variety testing system that evaluates variety performance is carried out over 2 harvest years without grazing. Our data show that the major differences between varieties in persistence and DM yield were

not apparent until the third and fourth harvest year. This suggests that testing beyond two harvest years is necessary to identify appropriate varieties that are suitable for longer leys.

Table 1. Red clover and total (red clover + grass) dry matter yield (t ha^{-1}) of 12 red clover varieties and selection lines in harvest years 1 and 4. Data are derived from a total of 3 cuts in each harvest year.

Variety	Year 1		Year 4		Plant density (plants 0.25m^{-2})
	Red clover	Total	Red clover	Total	
Aa4557	16.2 (8)	21.5 (8)	3.54 (6)	17.52 (9)	22.7
Aa4559	19.5 (5)	24.9 (3)	9.93 (2)	20.14 (3)	23.3
Aa4560	14.2 (10)	19.1 (10)	3.22 (7)	18.79 (6)	13.8
Aa4561	15.5 (9)	19.8 (9)	2.59 (10)	16.99 (12)	9.9
Milvus	22.6 (1)	26.4 (2)	6.27 (5)	19.65 (5)	15.4
AberChianti	19.8 (4)	24.3 (5)	7.54 (4)	20.04 (4)	23.3
AberClaret	21.9 (2)	27.4 (1)	10.19 (1)	21.01 (1)	20.5
Pavo	21.3 (3)	24.5 (4)	7.62 (3)	20.20 (2)	14.6
Merviot	18.3 (6)	22.4 (7)	1.98 (11)	17.89 (8)	6.0
Britta	14.1 (11)	18.5 (11)	2.99 (8)	17.36 (10)	15.3
Vivi	7.5 (12)	13.6 (12)	1.54 (12)	17.27 (11)	6.3
Amos	17.6 (7)	23.2 (6)	2.76 (9)	18.14 (7)	6.5
s.e.d.	1.17	0.86	0.89	0.68	3.08
Sign.(*** P<0.001)	***	***	***	***	***

Conclusion

Evidence from these field experiments over four harvest years shows that some red clover varieties maintain a high DM yield into the fourth harvest year and the yield decline between harvest years 1 and 4 is considerably less than in some of the commercially available varieties. The decline in yield is attributed to loss of plant numbers within the sward. The results have implications for the duration of red clover variety testing systems and their relevance to the use of red clover in grassland agriculture.

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Developing an optimal sampling strategy to assess the quality of perennial ryegrass varieties on a national variety evaluation scheme

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Abstract

The Irish national variety evaluation scheme measures *in vitro* dry matter digestibility (DMD) and water soluble carbohydrate (WSC) concentration of perennial ryegrass (*Lolium perenne* L.) varieties. Evaluations are made over several years of sowing, each harvested in the two years post-sowing, in replicated trials which are cost- and resource-intensive. The aim was to identity opportunities to optimize resource allocation for assessing *in vitro* DMD and WSC. The DMD and WSC rankings of combinations of year of sowing, harvest years and replicate blocks at two conservation cuts were compared to 'definitive' conservation rankings. Initially, increasing years of sowing was the best strategy to reliably rank the quality of varieties; however, there was a decrease in the rate of gain for an additional year of sowing that resulted in using an additional harvest year or block as the next effective strategy. Overall, a sampling strategy would require a combination of years of sowing, harvest years and blocks to account for inherent variation, experimental error and genotype × environment (G × E) interactions.

Introduction

The Irish national evaluation programme for perennial ryegrass varieties assesses DMD and WSC under a combined grazing and conservation management. At present both DMD and WSC are reported as mean values from all harvests taken in the year. Burns *et al.* (2013) recommended reporting both DMD and WSC as separate conservation and grazing values, rather than one annual value, as an acceptable balance between providing accurate, relevant information to the end-user while maintaining an accessible format.

For the purposes of evaluation schemes the relative performance (i.e. ranking) of varieties is more important than the absolute values as these are particular to the test site and conditions. Thus, rank changes are of primary concern to variety evaluation schemes as they provide an indication of the consistency of ranking (Conaghan *et al.*, 2008). Evaluation of quality is carried out throughout the year to ensure that the conditions during the assessment of varieties are representative of the target (i.e. on-farm) conditions. Evaluation schemes are under a fixed annual reporting cycle with limited resources to achieve their aims. As these constraints limit the amount of data that can be utilized for making recommendations, important decisions need to be taken regarding the optimal allocation of resources. The objective was to assess the influence selection criteria (year of sowing, harvest year, and replicate blocks) had on the conservation harvests rankings of *in vitro* DMD and WSC.

Material and methods

Fifteen perennial ryegrass varieties (6 intermediate- and 9 late-heading) were sown in two maturity group trials based on the heading date of each variety, in each of three years (2001, 2005, 2006) in a randomized complete block design (4 blocks). Each plot was harvested in the two subsequent years following sowing under a 6-cut combined simulated grazing and conservation management. At the conservation cuts (cut 2 and 3; hereafter referred to as 'Silage 1' and 'Silage 2') a *c.* 300 g sub-sample was taken from each harvested plot and assessed for DMD and WSC using NIRS. The 'definitive' conservation value and rank for DMD and WSC was calculated as per Burns *et al.* (2013). To assess the effect of selection criteria (year of sowing, harvest years and block), a mean DMD or WSC value per variety was calculated for each combination of selection criteria. All permutations of each of the selection criteria were assessed; for example, one year of sowing with one harvest year for two blocks had each individual year of sowing (2001, 2005, 2006) assessed at each harvest year (one- or two-year old) for the six permutations of two blocks (1+2, 1+3, 1+4, 2+3, 2+4, 3+4). This resulted in a total of 18 different permutations of 1 year of sowing, 1 harvest year and 2 blocks. The rank order was calculated within each maturity group for both DMD and WSC at both 'Silage 1' and 'Silage 2', and Spearman rank correlations were carried out between each of these permutations with their respective 'definitive' conservation rank. The mean Spearman rank correlation of all permutations was calculated and the standard error of this average was calculated and pooled across maturity groups. This process was repeated for all combinations of selection criteria.

Results and discussion

Overall, many of the correlations were weak between combinations of selection criteria and the respective 'definitive' conservation rank for both DMD and WSC.

Table 1. Mean Spearman rank correlation (r) of combinations of selection criteria correlated with 'definitive' conservation ranking for WSC and *in vitro* DMD

Selection Criteria			WSC				*In vitro* DMD			
			Silage 1		Silage 2		Silage 1		Silage 2	
Years of sowing	Harvest Years	Blocks	r	SEM	r	SEM	r	SEM	r	SEM
1	1	1	0.411	0.06	0.297	0.07	0.181	0.07	0.354	0.07
1	1	2	0.350	0.05	0.329	0.05	0.474	0.04	0.288	0.05
1	1	3	0.519	0.05	0.459	0.02	0.721	0.02	0.444	0.09
1	1	4	0.598	0.08	0.528	0.12	0.623	0.12	0.458	0.14
1	2	1	0.501	0.08	0.347	0.08	0.711	0.06	0.426	0.10
1	2	2	0.544	0.05	0.509	0.05	0.756	0.03	0.519	0.07
1	2	3	0.601	0.05	0.554	0.06	0.786	0.02	0.633	0.07
1	2	4	0.587	0.08	0.586	0.09	0.815	0.04	0.610	0.13
2	1	1	0.511	0.04	0.340	0.05	0.685	0.04	0.438	0.05
2	1	2	0.586	0.03	0.477	0.03	0.754	0.01	0.508	0.04
2	1	3	0.630	0.03	0.581	0.03	0.789	0.01	0.570	0.04
2	1	4	0.676	0.03	0.619	0.05	0.814	0.02	0.576	0.07
2	2	1	0.596	0.07	0.463	0.06	0.767	0.04	0.573	0.09
2	2	2	0.646	0.04	0.599	0.04	0.826	0.02	0.697	0.05
2	2	3	0.706	0.04	0.664	0.03	0.848	0.01	0.731	0.05
2	2	4	0.693	0.06	0.719	0.05	0.848	0.04	0.821	0.06
3	1	1	0.603	0.07	0.389	0.07	0.716	0.04	0.526	0.08
3	1	2	0.650	0.05	0.543	0.04	0.798	0.02	0.642	0.04
3	1	3	0.756	0.04	0.655	0.03	0.829	0.02	0.702	0.05
3	1	4	0.730	0.09	0.723	0.05	0.843	0.03	0.738	0.07
3	2	1	0.632	0.09	0.536	0.08	0.832	0.03	0.614	0.19
3	2	2	0.726	0.06	0.651	0.05	0.840	0.02	0.753	0.06
3	2	3	0.771	0.04	0.719	0.07	0.849	0.02	0.774	0.06
3	2	4	0.771	*	0.723	*	0.883	*	0.876	*

Correlations were higher for 'Silage 1' than 'Silage 2' with two exceptions (Table 1). The many low correlations may reflect the 15 varieties being from elite germplasms, such that varietal differences in quality traits may be relatively small and the small scale of experimental error may be sufficient to permit a significant re-ranking. G × E interactions have been reported in

evaluation schemes (Conaghan *et al.*, 2008) and weak correlations may suggest that evaluation has occurred over a wide range of environmental conditions that are representative of conditions on Irish farms. Another potential consideration is the relative maturity of varieties at harvest. For pragmatic reasons, all varieties within a maturity group are harvested on the same day. Therefore, there is the possibility that varieties were assessed at varying stage of maturity in each year, differentially affecting some varieties.

Increasing the number of years of sowing from one to two increased the correlation with the 'definitive' rankings by 0.153 and 0.112 for DMD and WSC respectively. Adding an additional year of sowing increased the correlations further but at a lower rate of increase (0.065, 0.067 for DMD and WSC respectively). An additional harvest year increased the correlation with the definitive rankings for both DMD and WSC, but to a lesser extent than an additional year of sowing (0.1385, 0.079 for DMD and WSC respectively). For both WSC and DMD increasing the number of replicate blocks increased the correlation with the 'definitive' conservation rank, whereby the rate of increase was smaller for each additional block. The use of blocks as a form of replication is important as these provide an assessment of the variation within varieties and improves the confidence with which a statistical comparison between varieties can be made. Therefore, for any sampling strategy, two blocks would be a minimum requirement to allow statistical inferences between varieties to be made.

Financial and time implications of choosing selection criteria must also be considered. For example, evaluating additional year of sowing or harvest years requires the same time-frame but an additional year of sowing is more costly than an additional harvest year due to the cost of the re-sowing process. Furthermore, on farm, grass swards have a longer life cycle than two years, and additional harvest years may be an important indicator of the long term performance of the sward. Another consideration when assessing several quality traits is the relationships between those traits. A loss in performance in one trait may be compensated for by an improvement in another. This would be of particular interest to grass breeders who aim to obtain improved quality traits without a negative trade-off in other traits.

In conclusion, inherent variation, experimental error and G × E interactions influence the quality ranking of perennial ryegrass varieties. To account for these factors, a combination of years of sowing, harvest years and replicate blocks is required to provide a reliable and robust assessment. A sampling strategy would require a minimum of 2 blocks to allow for statistical comparisons between varieties. Initially an additional year of sowing would be the best strategy to improve the reliability of quality rankings; however, subsequently either an additional block or harvest year would improve reliability more than an additional year of sowing.

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Screening reveals opportunities for high sugar cultivars of *Lolium perenne* L.

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Abstract

Tetraploid cultivars of perennial ryegrass (*Lolium perenne*) contain higher levels of water soluble carbohydrates (WSC) than diploid cultivars, which is advantageous for animal nutrition, milk composition and the reduction of N-emissions. However, due to reduced tillering, their swards are not as dense as those of diploid cultivars and are therefore less suitable for grazing. In a field experiment, 63 cultivars of perennial ryegrass and one cultivar of *xFestulolium loliaceum* were screened for WSC content in autumn 2010. The tetraploid group exhibited higher ($P < 0.001$) herbage WSC contents (142 g kg^{-1} DM) than the diploid group (124 g kg^{-1} DM). Two diploid 'high sugar grass' cultivars (HSGs), especially bred for high WSC content, contained WSC (140 g kg^{-1} DM) comparable to the tetraploid group ($P = 0.72$). It is concluded that diploid HSGs can reach the levels of sugar content of tetraploid cultivars and, thus, provide both dense swards for grazing and the advantages of high WSC forage with respect to animal nutrition, milk production and the environment.

Keywords: *Lolium perenne*, sugar, WSC, variety

Introduction

A high content of water soluble carbohydrates (WSC) in the forage can reduce N-losses caused by urinary excretion and increase milk-protein yield (Miller *et al*., 2001; Staerfl *et al*., 2013), as compared to low-WSC diets. In certain cases a high WSC content may also increase dry matter intake (Moorby *et al*., 2006). In perennial ryegrass (*Lolium perenne* L.) tetraploid cultivars are known to contain more WSC than diploid ones (Gilliland *et al*. 2002; Salama *et al*., 2012). However, for grazing, agronomy may favour diploid cultivars, because their higher number of tillers provides a denser sward (Swift *et al*., 1993), which in turn may reduce sward damage and forage spillage due to fouling, especially under wet conditions. Therefore, it is important to know if there are diploid cultivars that reach the same levels of WSC as tetraploids, in order to meet the requirements of agronomy and animal nutrition, as well as the environment. For this purpose, in a field experiment, 63 cultivars of perennial ryegrass and one cultivar of *xFestulolium loliaceum* have been tested for WSC content.

Materials and methods

Field plots (6 m × 1.5 m) of 63 cultivars of perennial ryegrass and one cultivar of *xFestulolium loliaceum* were sown at Zurich-Reckenholz (47° 26' N 8° 30' E, 440 m a.s.l., mean annual temperature: 9.4 °C, mean annual precipitation: 1031 mm) in spring 2009 at a rate of 22 kg ha^{-1}, corrected for germination ability. Of the 63 perennial ryegrass cultivars, 26 were diploid and 36 were tetraploid. Additionally, two diploid cultivars known as 'high-sugar' grasses (HSGs) were included in the test. From each plot, 200 g samples of fresh herbage were taken at a 5 cm cutting height at dawn, noon and dusk in late October 2010. The samples were treated in a microwave oven in the field immediately after cutting in order to stop enzymatic activity and were subsequently dried at 55 °C for 48 h. The samples were ground with a cutting mill (RetschSM1, Retsch, Germany) using a 0.75 mm sieve. Extraction and subsequent analysis with anthrone (Fischer, 1998), modified according to Trethewey and Rolston (2009), was conducted after purification with chloroform (Bligh and Dyer, 1959). For this study, the dawn, noon and dusk WSC values, expressed as glucose equivalents, were pooled into a mean daily value. The experimental design was a Latin rectangle with four rows and four columns.

Diploid, tetraploid and HSGs were compared by contrasts following the analysis of variance (ANOVA) using the R statistical software package (version 2.15.2).

Results and discussion

The tetraploid cultivars (Figure 1) exhibited markedly ($P < 0.001$) higher contents of WSC sugars (142 g kg^{-1} DM) than diploid cultivars (124 g kg^{-1} DM). The two HSGs, #32 and #18, showed the highest WSC of all non-tetraploid cultivars (140 g kg^{-1} DM). Their WSC contents were significantly ($P < 0.01$) higher than the mean content of the diploid cultivars but did not differ ($P = 0.72$) from the tetraploid group. These findings from 64 cultivars confirm results obtained in an investigation with 12 cultivars (Gilliland *et al.*, 2002), in which the diploid HSG cultivars attained the WSC levels of tetraploid perennial ryegrass cultivars. Although distinct differences between ordinary diploid cultivars and HSGs were detected in our autumn screening, spring and summer harvests should also be assessed in order to get to an all-encompassing overview.
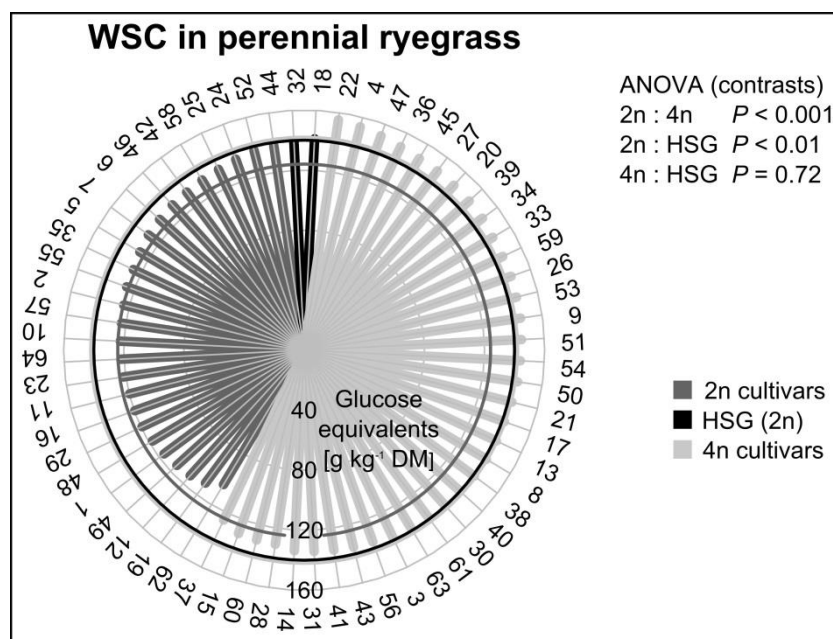
Figure 1. Content of water soluble carbohydrates (WSC) expressed as glucose equivalents (g kg^{-1} DM) in herbage of an autumn regrowth of 63 cultivars of *Lolium perenne* L. and one cultivar of *xFestulolium loliaceum* cut at 5 cm. Groups of common tetraploid, 4n (light grey) and diploid, 2n (dark grey), as well as 'high sugar' (HSG) diploid (black) cultivars. Bold circles indicate means.

Interestingly, the diploid cultivars #44, #52 and #24, which are not specifically marketed as HSGs, had high contents of soluble sugar, similar to those of the two HSGs ($P = 0.65$), revealing a certain potential for selection of new high-sugar cultivars from common diploid material. In the variety trials for the Swiss list of recommended varieties (data not shown), which contained the cultivars of the experiment described above, both the diploid HSGs (ground cover of 72% at a 14 cm row spacing) and the ordinary diploid cultivars (ground cover of 75%) had significantly (both $P < 0.001$) denser swards than the tetraploid group (ground cover of 55%). The data also indicate that the ground cover of diploid HSGs does not differ ($P = 0.49$) from that of the ordinary diploid cultivars.

Conclusion

We conclude that diploid HSGs can reach the levels of WSC of tetraploid cultivars while providing sward characteristics similar to other diploid cultivars. Thus, they may offer both

good sward characteristics for grazing and the advantages of high-sugar forage regarding animal nutrition, milk-protein and the environment.

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The influence of autumn closing date and spring opening date on herbage production and quality in spring and throughout the growing season

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Abstract

In perennial ryegrass swards, leaf senescence rate increases during the winter period, while leaf extension rate declines, resulting in a reduction in herbage mass and quality. The objective of this experiment was to examine the effect of 4 autumn closing dates and 2 spring opening dates on herbage yield and chemical composition over two full grazing seasons, with particular focus on the early spring period. Each day delay in closing date from 1 Oct to 15 Nov reduced opening herbage accumulation by 13.4 kg DM/ha. Each day delay in opening date from 1 Feb to 1 March resulted in 6.8 kg DM/ha of herbage accumulation. Opening plots in February had no effect on cumulative spring herbage production and resulted in herbage with a higher proportion of leaf and a higher concentration of crude protein and dry matter digestibility when compared to opening in March. There was no significant difference in total yearly herbage yield between the plots closed in October and plots closed in November.

Keywords: perennial ryegrass, yield, quality, winter growth

Introduction

During the winter period, the growth of perennial ryegrass (PRG; *Lolium perenne* L.) is based on a reduction in leaf extension and an increase in leaf senescence rate (Hennessy *et al.*, 2008). As a result, the net accumulation of grass during the winter is low, particularly as the sward reaches a ceiling in herbage yield (Ryan *et al.*, 2010). The final autumn grazing date (closing date) has a large influence on the yield and quality of grass available for early spring grazing (Ryan *et al.*, 2010). As closing date is delayed in the autumn, herbage accumulation is reduced in early spring by up to 15 kg DM/ha per day (O'Donovan *et al.*, 2002). The initial spring grazing date (opening date) will also influence the yield and quality of herbage available in the first and subsequent rotations (O'Donovan and Delaby, 2008). The objective of this experiment was to examine the effect of four autumn closing dates and two spring opening dates on herbage yield and chemical composition over two full growing seasons

Materials and methods

The experiment was conducted at the Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland (52° 16' N 8° 25' W), from 15 October 2011 for two years. A one-year-old PRG sward (0.79 PRG) was divided into 24 plots (3 m × 5 m). The study was a randomized block design with a 4 × 2 factorial arrangement of treatments and included three replicates. The experiment was repeated over two consecutive growing seasons. The treatment factors included four autumn closing dates (CD1: 1 October, CD2: 15 October, CD3: 1 November and CD4: 15 November) and two spring opening dates, (ODE: 1 February, ODL: 1 March). Cumulative spring herbage yield was the sum of herbage production from opening date until the end of April. Cumulative summer herbage yield was herbage production from May until late August. Autumn herbage accumulation was the herbage yield at closing date. Each treatment received 215 kg/ha of fertilizer N over the growing season. All plots were harvested with an Etesia rotary blade mower (Etesia UK Ltd., Warwick, UK) to a stubble height of 4 cm. All mown herbage from each plot was collected and weighed, and a 0.1 kg subsample was dried for 48 h at 40ºC to determine dry matter (DM) percentage and to calculate yield in

terms of DM/ha. The dried sample was milled through a 1 mm sieve and analysed for dry matter digestibility (DMD) and crude protein (CP) content using near infra-red spectrometry (NIRS, Model 6500, FOSS-NIR System, 3400 Hillerød, Denmark) and the equation developed by Burns *et al*. (2010). Prior to harvesting at each closing and opening date, a sample of PRG tillers were collected by cutting them to ground level using a blade. A 40 g subsample was then cut at 4 cm above the base of the tiller (representative of the plot defoliation height), and the leaf blades, stem (total true stem and pseudostem) and dead components were sorted above the 4 cm stubble height. The separated fractions were dried for 16 h at 90°C for DM determination. Data were analysed using the mixed model procedure in SAS *v*9.3. Model terms included CLD, OD, CLD×OD, replicate and year.

Results and discussion

There was an interaction when DMD was examined (Table 1). Digestibility of herbage increased from CD1 to CD3 when plots were defoliated on ODE; however, when plots were defoliated on ODL there was no effect of CD on DMD.

Table 2. The effect of closing date on total DM yield and sward morphology the following spring.

OD	01 Feb				01 Mar				Significance			
CD	01Oct	15Oct	01Nov	15Nov	1Oct	15Oct	01Nov	15Nov	S.E.	CLD	OD	INT
Open yield	1207^{Ax}	1023^{Bx}	671^{Cx}	693^{Cx}	1467^{Ay}	1267^{By}	883^{Cy}	752^{Cy}	84.2	***	*	N.S.
Total yield	9726^{A}	10814^{B}	10230^{AB}	10378^{AB}	10226^{A}	10439^{B}	10679^{AB}	10291^{AB}	287.3	*	N.S	N.S.
DMD g/kg	808^{a}	817^{b}	816^{b}	812^{ab}	795^{c}	797^{c}	804^{c}	805^{ac}	2.0	***	***	*
CP	0.23^{Ax}	0.24^{Ax}	0.25^{Bx}	0.25^{Bx}	0.22^{Ay}	0.21^{Ay}	0.20^{By}	0.22^{By}	0.002	***	***	N.S.
Leaf >4cm	0.37^{Ax}	0.44^{BCx}	0.42^{Bx}	0.44^{Cx}	0.33^{Ay}	0.37^{BCy}	0.38^{By}	0.41^{Cy}	0.016	***	***	N.S.
Stem>4cm	0.21^{ABx}	0.21^{ABx}	0.18^{BCx}	0.15^{Cx}	0.28^{ABy}	0.26^{ABy}	0.24^{BCy}	0.23^{C}	0.019	*	***	N.S.
Dead>4cm	0.42^{A}	0.35^{B}	0.40^{AB}	0.41^{A}	0.40^{A}	0.36^{B}	0.38^{AB}	0.36^{AB}	0.033	***	N.S	N.S.

CD = Closing date, OD = Opening date, INT = Interaction of OD×CD, yield = opening or total yearly yield in kg DM/ha; Leaf >4cm = proportion of leaf above 4 cm stubble height, SE = Standard error, xy = within a row means with different superscripts differ significantly for OD; ABC = means with significant difference for CD; abc = means with significant difference for OD×CD;
N.S = not significant, * = *P*<0.05, ** = *P*<0.01, *** = *P*<0.001

Each day delay in closing date from CD1 to CD4 reduced opening herbage mass by 13.4 kg DM/ha, which is intermediate between the 11 kg DM/ha reduction reported by Carton *et al.* (1988) and 15 kg reduction reported by O'Donovan *et al*. (2002). As a result, plots which were closed in October had a greater cumulative spring herbage yield than plots which were closed in November (Figure 1). The effect of CD on cumulative herbage production during the spring period was largely due to the difference in herbage accumulation from CD to OD, as CD had no effect on herbage production in April. Plots which were opened in March accumulated an additional 194 kg DM yield, or 6.8 kg /ha per day more (*P*<0.05), than ODE plots; however, in April plots which were opened in February yielded 111 kg DM/ha more herbage than ODL plots (*P*<0.05); as a result OD had no significant effect on cumulative spring herbage production. There was no effect of CD or OD on summer herbage production. In the autumn period, each day delay in closing from CD1 to CD3 increased herbage availability by 17.4 kg

DM. There was no significant difference in autumn herbage yield between CD2 and CD4, after an additional 31 days of growth. This suggests a ceiling in autumn herbage accumulation was reached between CD2 and CD3, similar to that described by Hennessy *et al.* (2008). There was no significant difference in total herbage yield between plots which were closed in October and those closed in November.

The concentration of CP in the spring was higher in herbage that was closed after CD2. Hennessy *et al.* (2008) reported an increase in CP concentration with later closing dates as a result of increased leaf and reduced stem and dead proportions in the sward. Similarly, in the present experiment the proportion of leaf was higher in swards which were defoliated after CD1 as the proportion of stem decreased. An increase in the proportion of leaf indicates an increase in sward quality (Beecher *et al.*, 2013). There was a higher proportion of leaf and a lower proportion of stem upon opening at ODE than ODL. As a result, the concentration of CP and DMD was higher at ODE than ODL plots ($P<0.001$); however there was no residual effect of OD or CD on CP or DMD after opening.
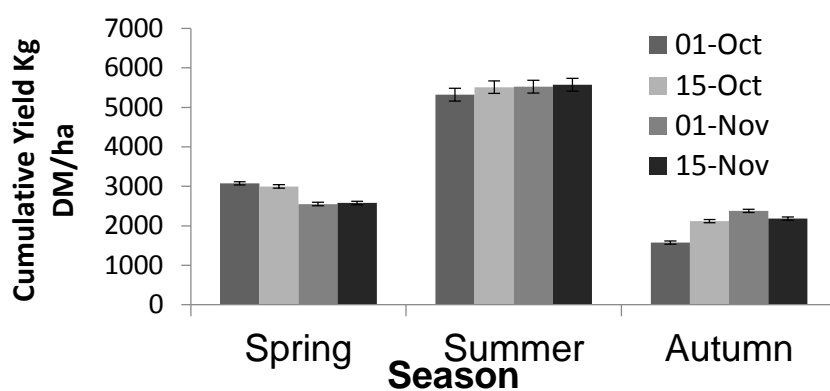
Figure 1. The effect of closing date on herbage yield during the entire growing season

Conclusion

By delaying closing date from 1 October to 15 November there was a 13.4 kg DM/ha per day reduction in opening herbage accumulation. Closing swards on 15 October maximized spring DM yield but did not result in any reductions in total herbage yield. Grazing from 1 February provides highly digestible grass with a high crude protein concentration, and has no effect on the cumulative herbage production over the spring period.

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Increasing protein yields from grassland by reseeding of legumes

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Abstract

According to the politically motivated protein initiative in Baden-Wuerttemberg, it was the objective of a field experiment in South Germany to increase protein yields of farm-grown roughage avoiding the use of genetically modified soya as a feed for dairy cows. Three different legumes were tested at two different locations. First, an intensively used grassland region of Oberschwaben, with high rainfall and the best growing conditions for grassland, and secondly, in the summer dry region Swabian Alb, with shallow soils and only marginal growing conditions. Lucerne (*Medicago sativa*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) were reseeded in permanent grassland swards with different sowing rates and different sowing dates. First results show better establishment of legumes by early seeding and subsequently higher yields of dry matter. Red clover was more successful than white clover and reseeding of lucerne.

Keywords: protein yield, permanent grassland, legumes, *Medicago sativa, Trifolium pratense*

Introduction

Consumers in Germany wish for sustainable food production without genetically modified consituents. For milk production, therefore, the use of imported soya seems to be limited in the future. It is possible that feed protein for milking cows can be produced as farm-grown roughage by better use of grasslands (Buchgraber, 2001). Increasing protein in permanent grassland can be maintained by, among other means, higher nitrogen fertilization, early cutting dates or increasing the percentage of legumes in the grassland swards. Moreover, higher proportions of legumes allow production of protein with lower support of fossil energy through using the symbiotic fixation of atmospheric nitrogen. The increase of farm-grown protein is a political target in Baden-Wuerttemberg, as in other states of Germany. Estimations show a potential of around 800 000 t protein from grassland and forage fields (Engel *et al.*, 2013). But high percentages of legumes are not easy to maintain because the nitrogen content of manure from intensive dairy farming is too high for the growth conditions of legumes. Additionally, red clover and lucerne are mainly used as legumes for arable field cropping and do not tolerate the frequent mowing of intensive grasslands. Therefore, in 2012 the agricultural institute Baden-Wuerttemberg (LAZBW), together with the University of Hohenheim, establsihed two field trials at two different locations. The objective was to increase the percentage of red clover, white clover or lucerne in permanent grassland via reseeding. First, the rates of emergence of the legumes are observed. Secondly, yields and protein delivery from the experimental sites and, moreover, from 10 fields on practical farms are measured. Here the results of seed emergence and results from the first experimental year are reported.

Materials and methods

In two grassland regions of Baden-Wuerttemberg (Oberschwaben: intensive grass production with 5 cuts; 1000 mm rainfall, 670 m a.s.l.; and Swabian Alb: shallow calcareous soils; 650 mm rainfall, 850 m a.s.l.) two experiments on permanent grassland were established.

In the split-plot-design (size of parcels 10m², 3 replications) the following treatments were compared (Table 1). As legume species, white clover (*Trifolium repens*), red clover (*Trifolium pratense*) and lucerne (alfalfa) (*Medicago sativa*) were reseeded on two dates (early: 19 June

at Oberschwaben and 4 July at Swabian Alb; and late: 23 August at Oberschwaben; 27 August at Sw.A.). Two seed rates were tested. In order to ensure optimum seed establishment conditions and better control of new seedlings, the permanent grassland swards were treated with a herbicide before seeding. Additionally, the grassland swards were opened by harrowing in order to allow soil contact of the seed. Legumes were seeded mechanically using the 'Vredo' slot seeder system.

Table 1. Treatments of legume seed rates, species, varieties and sowing dates

Treatments	Factors	
Plants and seed rate	Control:	Control without reseeding
	WKL6:	white clover 6 kg ha^{-1} (varieties: Riesling and Merlyn)
	WKL15:	white clover 15 kg ha^{-1}
	RKL10:	red clover 10 kg ha^{-1} (varieties: Milvus and Merula)
	RKL20:	red clover 20 kg ha^{-1}
	LUZ10:	lucerne 10 kg ha^{-1} (varieties: Daphne and Sanditi)
	LUZ20:	lucerne 20 kg ha^{-1}
Seed date	Early:	in region Oberschwaben 19.06.2012
		in Jura (Swabian Alb) 04.07.2012
	late:	Oberschwaben 23.08.2012
		Swabian Alb 27.08.2012

In order to control the effect of reseeding, the emergence of seedlings was observed 5 weeks after sowing and categorized with an evaluation scale of 1-5 (Table 2). Dry Matter (DM) and protein yields were investigated from the beginning of the second experimental year (2013).

Table 2. Scoring scale for the evaluation of the reseeded legumes

1	no	no seedlings visible
2	few	few seedlings visible
3	medium	various seedlings visible
4	strong	scattered rows of seedlings visible
5	very strong	various rows of seedlings likely to be visible

Results

An overview of seed emergence of the reseeded legumes is presented in Table 3. It is clear that the early sowing date was more successful at both locations. The late-sown seed was not well established, especially at the Swabian Alb location. This is in line with results reported from Black (*et al.*, 2006) in New Zealand, where spring seeded ryegrass-white clover mixtures show better yields than later seed dates in the year. Grabber showed similar results for red clover and also mentioned the effects of seeding dates (Grabber, 2009). The comparisons of seed rates show better seed emergence with the higher seed rates. Red clover, especially, was well established and scored best, whereas white clover and lucerne had problems with establishment. The late seeding date of lucerne had an advantage only in the intensive grassland area of Oberschwaben.

Table 3. Evaluation of reseeded legumes in field trials in Oberschwaben and Swabian Alb (scale from 5 = fully closed rows of legumes to 1 = no legumes visible (see Table 2))

			Site and sowing date			
			Oberschw.	Oberschw.	Sw.Alb	Sw.Alb
	Treatment	Seed rate (kg ha^{-1})	early	late	early	late
1	control	0	0 e	0 e	0	0 b
2	w.clover 6	6	3.3 cd	3.3 d	3.7 c	0.7 b
3	w.clover 15	15	4.3 ab	4.0 c	4.3 b	1.7 a
4	r.clover 10	10	5.0 a	4.3 bc	4.0 bc	0.7 b
5	r.clover 20	20	5.0 a	5.0 a	5.0 a	1.7 a
6	lucerne 10	10	2.7 d	3.0 d	3.7 c	0.3 a
7	lucerne 20	20	3.7 bc	4.7 ab	3.7 c	1.0 b

The percentages of legumes varied independently of the seed rate of establishment. At both the grass-rich swards in Oberschwaben, and the more herb-rich grassland in Swabian Alb, the early sowing dates mostly resulted in higher percentages of legumes in the first experimental year (Table 4). Proportions of red clover were higher than those of either lucerne or white clover.

Table 4. Average proportion of legumes (%) of each treatment of the experiments in Oberschwaben and Swabian Alb – average of all growths in 2013

			Site and sowing date			
			Oberschw.	Oberschw.	Sw.Alb	Sw.Alb
	Treatment	Seed rate (kg ha^{-1})	early	late	early	late
1	control	0	0.97 c	1.2 b	8.3 e	5.0 b
2	w.clover 6	6	7.9 b	1.4 b	13.9 ce	3.3 b
3	w.clover 15	15	8.3 b	2.4 b	17.8 c	4.3 b
4	r.clover 10	10	17.4 a	4.3 ab	28.9 b	6.6 b
5	r.clover 20	20	20.3 a	9.1 a	37.8 a	12.8 a
6	lucerne 10	10	1.44 c	1.5 b	11.7 de	3.7 b
7	lucerne 20	20	1.9 c	2.9 b	14.4 cd	4.4. b

Conclusion

In the future it will be necessary to use the potential of legumes to produce protein-rich roughage in order avoid using high rates of mineral-N fertilization and thereby reduce N_2O emissions. A profitable objective for modern grassland management is therefore to increase the amounts of legumes in order to get higher protein yields, higher DM-yields and better forage values. This leads at the same time to a reduction of nitrogen emissions and lower energy consumption. The first results, after introduction of national strategies, show that reseeding with legume seeds increases the percentages of legumes, and that red clover has better chances for establishment in permanent grassland than white clover or lucerne. Early seed sowing dates seem to work better than late seeding, and protein yields were higher in the early seeded treatments. Moreover, the establishment of lucerne, which is usually used only in arable field

cropping systems, showed positive effects. It is assumed that a frequent reseeding of red clover and lucerne can be also a way to increase protein yields of grassland.

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Change in birdsfoot trefoil (*Lotus corniculatus* L.) nutritive value with stem elongation, flowering and pod formation

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Abstract

Birdsfoot trefoil (BFT) grazed in pure stands supports rapid gain and high milk production in ruminants compared with grass pastures, and retains high nutritive value when stockpiled. In this study, the change in forage nutritive value from 4 to 12 weeks of mid-summer regrowth was assessed. Rapid stem elongation occurred until 7.5 weeks, and new internodes were added at stem tips through 15 weeks. At 12 weeks of regrowth, crude protein (CP), acid detergent fibre (ADF) and amylase-treated neutral detergent fibre (aNDF) were 199, 292, and 348 g kg^{-1} dry matter (DM), respectively; values that are associated with 'good' quality lucerne hay. However, total digestible nutrients (TDN), an indicator of energy availability for ruminant production, was 620 g kg^{-1} DM at 12 weeks of regrowth, higher than TDN values reported for 'premium' lucerne hay.

Keywords: *Lotus corniculatus*, nutritive value, ruminant production, total digestible nutrients

Introduction

Birdsfoot trefoil contains sufficient condensed tannin to precipitate excess plant protein in the rumen, preventing bloat and reducing ammonia synthesis from protein deamination and lowering urinary nitrogen. In the abomasum, pH-mediated protein release from BFT tannins allows digestion of plant protein and absorption of amino acids (Waghorn *et al*., 1987). High levels of ruminally undegradable protein result in higher liveweight gain on BFT than lucerne (Douglas *et al*., 1995); higher non-fibrous carbohydrate (NFC) in BFT compared with lucerne may also contribute to higher ruminant productivity (MacAdam and Griggs, 2013). Rotational stocking at the bloom stage is recommended for long-term productivity of BFT (Undersander *et al*., 1993). In this study, the nutritive value of BFT was determined at the first bloom, full bloom, pod formation and pod maturity stages of a single regrowth cycle.

Materials and methods

Ten individual plants of 'Norcen' BFT were grown in 11.4-L pots containing a medium of vermiculite, bark, peat moss, perlite and nutrients. Plants were started from seed on 3 April 2011 in a heated greenhouse with 54 mol m^{-2} d^{-1} supplemental lighting. Initial growth was cut to a 75-mm stubble on 7 June 2011 and plants were moved to an outdoor bench in Logan, UT (41.74° N 111.83° W) until autumn. After overwintering outside, the eight surviving plants were returned to the greenhouse in early April 2012. Initial growth was cut to 75 mm on 7 June 2012 and plants were again grown on an outside bench until autumn. In both years, plants were irrigated to field capacity daily with water containing 0.5 g L^{-1} of 210 g kg^{-1} N, 22 g kg^{-1} P and 166 g kg^{-1} K. In 2012 at 4, 6, 8, and 12 weeks of regrowth, on 4 and 18 July and 3 and 30 August, one-quarter of each plant was harvested, dried at 55 °C to constant weight, and ground to pass the 1 mm screen of a cutting mill. Forage nutritive value was determined using near-infrared reflectance spectroscopy. A mixed grass-legume hay equation developed according to procedures of Shenk and Westerhaus (1991) from a calibration set containing multiple legume species including BFT was used to predict sample composition. Calibration data were obtained as ADF, acid detergent lignin, aNDF, and CP according to AOAC (Latimer, 2012); *in vitro* true DM digestibility (IVTDMD, 48-hr incubation) according to Goering and Van Soest

(1970); NDF digestibility (NDFD) was calculated from aNDF and IVTDMD, NFC (DM - (ash + CP + ether extract + aNDF), and TDN according to the 2001 NRC Dairy summative equation (NRC, 2001). Changes in BFT forage quality parameters over 12 weeks of regrowth were described with linear regression for lignin, or with linear regressions on ln (x)-transformed data for other variables, using Excel version 14.3.1 (Microsoft 2011, Seattle, WA USA). Significance of linear regressions was evaluated using StatPlus:mac LE version 2009 (AnalystSoft Inc., Alexandria, VA).

Results and discussion

Following removal of herbage to 75 mm, stem growth was initiated from axillary buds on the remaining stubble and stem internode number produced a sigmoid growth pattern (Fig. 1).
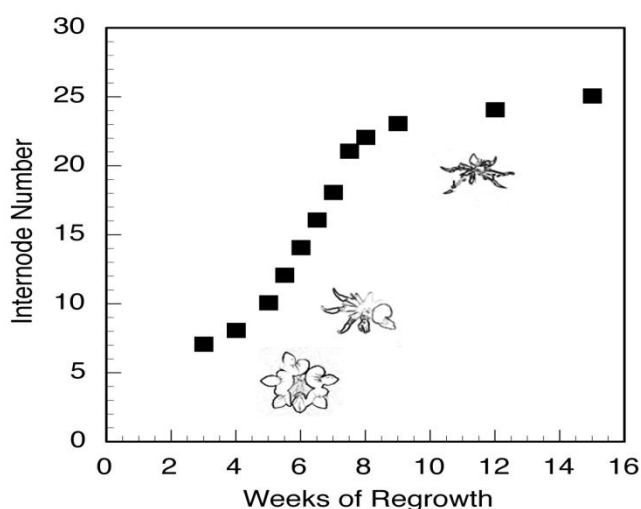
Fig.1 Internode number was recorded for 10 BFT plants from 28 June through 21 September 2011. Drawings indicate periods of full flowering, pod formation and seed maturation at 6, 8 and 12 weeks of regrowth, respectively.

The end of linear stem growth at 7.5 weeks coincided with the beginning of seed fill. During regrowth, CP, NDFD, TDN, NFC, and IVTDMD all declined while lignin and ADF increased, as would be expected (Table 1).

Table 1. Forage nutritive value variables at 4, 6, 8 and 12 weeks of BFT regrowth (n = 8). *P*-values are for regressions; means separations are indicated by different letters within rows.

Weeks of midsummer regrowth	4		6		8		12		*P* values
Crude protein (g kg^{-1} DM)	267	a	210	b	213	b	199	b	< 0.01
Acid detergent fibre (g kg^{-1} DM)	196	c	239	b	272	a	292	a	< 0.01
Amylase-treated neutral detergent fibre (g kg^{-1} DM)	221	c	276	b	322	a	348	a	< 0.01
NDF digestibility, 48-hr (g kg^{-1} NDF)	457	a	370	b	333	c	338	c	0.01
Acid detergent lignin (g kg^{-1} DM)	34	d	39	c	46	b	54	a	< 0.01
Total digestible nutrients (g kg^{-1} DM)	716	a	682	b	642	b	620	b	< 0.01
Non-fibrous carbohydrates (g kg^{-1} DM)	437	a	451	a	408	b	388	c	0.03
In vitro true DM digestibility, 48 hr (g kg^{-1} DM)	879	a	826	b	785	c	769	c	< 0.01

The forage nutritive value of oven-dried herbage will be higher than for the same material dried and baled in the field. However, when compared with the nutritional characteristics reported for lucerne hay by quality category (Robinson, 1998), values for TDN were significantly higher for BFT than for lucerne with comparable CP, ADF and NDF values. The components of TDN are NFC, CP, fatty acid concentration, and digestible NDF (NRC, 2001), and MacAdam and Griggs (2013) also reported higher NFC for BFT than lucerne for 2 years of field data. These data support the results of Collins (1982) who reported autumn average daily cattle gains of 1.1 to 1.4 kg on stockpiled BFT-Kentucky bluegrass (*Poa pratensis* L.) pastures.

Conclusions

High nutritive value is maintained in maturing BFT, including high NFC and TDN, which may contribute to the high liveweight gains of beef cattle grazing stockpiled BFT.

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Studies on forage quality of weed species in subalpine meadows in the Southeastern Carpathians of Romania

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Abstract

Experiments were conducted in the Bucegi Mountains, at 1800 m altitude, in the sub-alpine level of mountain pine (*Pinus mugo*), on a degraded pasture of *Nardus stricta* in a proportion of 40-60%. After 18 years of pasture improvement through liming and chemical and organic fertilization, under cow grazing system, there was a very high increase in dry matter (DM) and milk production, and *Nardus stricta* declined to disappearance. In contrast, after improvements, some species from the category of so-called weed species have proliferated, such as: *Deschampsia caespitosa* (4-12%), *Polygonum bistorta* (10-15%) and *Ligusticum mutellina* (6-10%) for which the chemical analysis was carried out. In conclusion, as a result of improvement works, these species have a high forage value and are finally fully consumed by dairy cows. Over a period of 18 years, on invaded variants of these 'weed' species, on average, 4.70 t DM ha^{-1} and 3180 L of milk per ha were obtained during 85 grazing days, due to the forage quality of the grassy carpet.

Keywords: grassland, *Nardus stricta* species, improvement, weed invasion, forage quality.

Introduction

The most common Carpathian Mountain pastures, with degraded grassy carpet, are invaded by *Nardus stricta* (Marusca *et al.*, 2010). After their improvement by various methods, *Nardus stricta* species is replaced by other more valuable species, including some so-called weeds, which usually are considered worthless. In several cases it was found that the 'weeds' actually have high forage value because of their content in nitrogen, phosphorus, potassium, *etc.* and valuable organic substances (Vintu *et al.*, 2009).

In long-term experiments in the Bucegi Mountains, there was a proliferation of several species of so-called weeds on some of the most efficient variants, leading to improvement of degraded *Nardus stricta* grasslands, and where was obtained the largest production of DM and cow milk yield per hectare. The question is, whether to destroy these 'weeds' or to keep them? The answer to this question we need information on the feed value of these plants, the main objective of this paper.

Materials and methods

Experiments on methods of improving degraded subalpine *Nardus stricta* grasslands were carried out in the Bucegi Mountains, at 1800 m altitude, since 1995.
On permanent grassland, where *Nardus stricta* has an initial contribution of 40-60%, liming was applied at 7.5 t ha^{-1} CaO, to correct soil acidity, and fertilizers (N150, P50, K50 kg ha^{-1}), on three consecutive years, followed by dairy cows paddocking (1 cow / 6 sq m / 5 nights) from 6 to 6 years. In 2013, after 18 years of the first intervention with chemical fertilizers, and followed by organic fertilizers with paddocking method, green biomass samples were taken in June, July, August from species *Nardus stricta, Deschampsia caespitosa, Polygonum bistorta, Ligusticum mutellina* and also for *Trifolium repens*, as control species for the qualities of forage. The first two species were collected from unimproved and improved grassland, and the next three species from improved plots where they were better represented.
Green samples were chopped and measurements were carried out by near infrared spectroscopy - NIRS. After drying and milling the samples were used to determine the total nitrogen, crude

protein (CP), by the Kjeldahl method, crude fibre (CF), the cell wall (NDF, ADF) by Van Soest method and the organic matter digestibility (OMD) coefficients in the NIRS. For the assessments, the average of the three samples collected at different stages of vegetation were taken into account, these not showing large differences in the results.

Results and discussion

Chemical composition and digestibility coefficients of organic matter of *Nardus stricta* and *Deschampsia caespitosa* species for unimproved and improved alternatives are shown in Table 1.

Table 1. Influence of grassland improvement on the organic matter content of *Deschampsia caespitosa* and *Nardus stricta* species

Species	Variant of improvement	Content, (%)				
		CP	CF	ADF	NDF	OMD
Nardus stricta	1. Unimproved	11.9	39.7	42.0	67.1	47.4
	2. Improved	12.9	36.8	39.6	63.7	50.7
	%, 2 compared to 1	108	93	94	95	107
Deschampsia caespitosa	1. Unimproved	8.3	36.9	41.4	67.8	46.8
	2. Improved	17.2	31.1	33.8	57.4	63.9
	%, 2 compared to 1	207	84	82	85	136

For *Nardus stricta*, there was no major differences in improvement of the content of crude protein, as it increased by only 1% compared to the unimproved version. Crude fibre content was decreased on average by 3% in the improved version, as in the case of cell wall content (ADF, NDF). The same difference of 3% was maintained for digestibility coefficients of organic matter, recording low values between 47-50%, indicating a low nutritional value to both.

Compared with *Nardus stricta,* for *Deschampsia caespitosa* a significant difference was recorded between the two versions, crude protein for the improved variant rising from a value of 8.3% to 17.2%. Crude fibre content decreased by 5% in the improved version. In the case of lignocelluloses (ADF), the values were lower by 9%. The same trend occurred for the NDF value, in this case a decline of 10% for the improved version.

Regarding the organic matter digestibility coefficients, difference between the two variants was 17% in favour of the improved variant (63.9%).

The chemical composition and organic matter digestibility coefficients of the three species of plant (*Trifolium repens, Polygonum bistorta* and *Ligusticum mutellina*) are shown in Table 2.

From the nutritional point of view, these three plant species have recorded similar values for their chemical element contents and the concentration of the cell walls.

The two species *Polygonum bistorta* and *Ligusticum mutellina* reached high levels of crude protein, with values between 24-25%, as compared to 28.7% for *Trifolium repens*. Note that all three species had low crude fibre content, about 18%. Their high nutritional value is confirmed by the low cellular constituent contents: ADF values between 22 and 25%, and NDF values between 30 and 32%. Organic matter digestibility coefficients, OMD, had high values. The highest value was obtained from *Trifolium repens* (72.9%) followed by the other two species with very similar values: 69.6% for *Polygonum bistorta*, and 69.8% for *Ligusticum mutellina*.

Table 2. The chemical composition of some species from improved plots

Species		Content, (%)				
		CP	CF	ADF	NDF	OMD
Trifolium repens	content	28.7	17.9	22.8	30.8	72.9
	%, relatively	100	100	100	100	100
Polygonum bistrorta	content	25.0	18.2	27.4	36.6	69.6
	%, relatively	87	102	120	119	95
Ligusticum mutellina	content	24.0	18.2	25.3	32.0	69.8
	%, relatively	84	102	111	104	96

For all the three species, determinations of the contents of phosphorus and potassium were made; values are shown in Table 3.

Table 3. The mineral element contents of some plant species of mountain improved grasslands

Species	Ash		Phosphorus		Potassium	
	Value	%	Value	%	Value	%
Trifolium repens	11.3	100	0.393	100	2.85	100
Polygonum bistorta	10.2	90	0.494	126	2.93	103
Ligusticum mutellina	10.4	92	0.632	161	3.51	123

In terms of the mineral composition of the two species *Polygonum bistorta* and *Ligusticum mutellina* have higher levels of phosphorus and potassium compared with *Trifolium repens*. *Ligusticum mutellina* species, with the highest values of P (0.632%) and K (3.51%) is distinguished.

Conclusion

As a result of improving the degraded sub-alpine grasslands of the Southeast Carpathians, after 18 years of experimentation, *Nardus stricta* species had a lower response to the improvement factors (liming, fertilization) in comparison with *Deschampsia caespitosa* which is consumed by animals and having better forage quality in these conditions. These plants are consumed mainly in the second half of the grazing season. After improvement works, the invasive species, referred to until now as 'weed species', *Polygonum bistorta* and *Ligusticum mutellina*, can be considered good forage, with a quality close to that of *Trifolium repens*, taken as the control species. In the specific conditions of the Carpathian sub-alpine zone, on improved grasslands, the *Deschampsia caespitosa*, *Polygonum bistorta* and *Ligusticum mutellina* species are consumed by dairy cows and can be considered as having medium to high forage value.

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Index of Authors

Goldringer I., 112
Golecký J., 553
Golińska B., 798, 816
Goliński P., 29, 142, 477, 759, 766, 798, 816
González A., 590
Görs S., 538
Gottardi S., 418
Gregis B., 786
Greve Pedersen M., 830
Griffith B., 239
Griffith G.W., 248
Griffith V., 279
Griffiths J.B., 233
Griggs T.C., 884
Grignard A., 641
Grinberg N.F., 826
Grogan D., 843, 870
Grosse Brinkhaus A., 593
Grygierzec B., 400
Guera K.C.S., 356
Gustavsson A.-M., 498
Gutiérrez R., 347
Gutmane I., 486
Hackett R., 583
Häfliger P., 321
Hahn M.A., 321
Hajšlová J., 495
Halasz A., 683
Hald A.B., 318
Harris P., 239
Harstad O.M., 553
Haş E.C., 887
Haughey E., 166
Hautier L., 376
Hayes R.C., 550
Hazard L., 112
Heard M.S., 254
Hecht J., 763
Hegarty M., 826
Heiermann M., 456
Hejcman M., 324, 327, 566
Hejcmanová P., 566
Helander M., 184
Helgadóttir Á., 15
Hennart S., 641
Hennessy D., 677, 737, 766, 783, 789, 792, 795, 810
Henry C.A., 541
Hensgen F., 477
Herremans S., 641
Herrmann A., 492, 535, 619

Herrmann C., 456
Herrmann K., 634, 638
Hetta M., 603
Hodkinson T., 438
Hoekstra N.J., 109, 166
Hofer D., 109, 166, 874
Hoffmann R., 282
Hoffstätter-Müncheberg M., 106
Hofstetter P., 658
Holshof G., 245, 544, 725
Horan B., 521
Hornick J.L., 181, 547
Hortová B., 421, 606
Huchon J-C., 559
Huguenin-Elie O., 695, 722, 756
Hujerová R., 327
Hummler T., 634
Humphreys M.W., 215
Huneau T., 559
Hunt S.R., 884
Husse S., 719, 722
Hutchings N.J., 97
Huws S.A., 550
Huyghe C., 29, 330, 743, 766, 807
Hyland J.J., 128
Iepema G., 665
Ineichen S., 680
Inostroza L., 858
Isselstein J., 103, 106, 160, 199, 367, 385, 743
Jakešová H., 495
Jamar D., 641, 776
Jan Ten Hagen P., 91
Jančová Ľ., 370
Jankowska J., 414
Jankowski K., 333, 407, 411
Janss L., 830
Jensen C.S., 830
Jensen J., 830
Jensen S.K., 600
Jilg T., 634, 638
Jiménez J.D., 587, 590, 622
Jokubauskaite I., 187
Jones D.L., 128, 273
Jones G., 743
Jones H., 651
Jones M., 438
Jørgensen M., 142
Jouany C., 112
Jovanovic M., 312
Juaristi A., 347
Julier B., 330

Julier Koubaiti B., 112
Kadziuliene Z., 468
Kaiser M., 385
Kanianska R., 370
Karabcová H., 391, 480
Karachristos C.N., 145
Karakosta C., 191
Karatassiou M., 157
Kasperczyk M., 400
Kauer K., 315
Kayser M., 106, 199
Kelly R., 852, 861
Kennedy E., 737, 877
Keres I., 315
Kerr S., 309
Kessler W., 3, 743
Keszthelyi S., 282
Kigongo J., 160
King R.D., 826
Kingston-Smith A.H., 849
Kipling R.P., 97
Kipparisides C., 462
Kirilov A., 647, 743
Kizeková M., 370
Klaas M., 438
Klotz C., 418
Klumpp K., 75
Kluß C., 492, 535
Kolczarek R., 407, 411
Kosmala A., 151
Kostopoulou P., 157
Koukoura Z., 462
Krstic D., 312
Krtková V., 495
Kryszak A., 333
Kryszak J., 333
Küchenmeister F., 103
Küchenmeister K., 103
Kurhak V., 453
Kusche D., 553
Kyriazopoulos A.P., 154
Laidna T., 315
Lalor S.T.J., 556
Lassalas J., 728
Látal O., 480
Lättemäe P., 780
Lausen P., 619
Lawrence D., 877
Lazaridou A., 157
Lazaridou M., 154, 157
Lea-Langton A.R., 465

Lebeau F., 631
Lee J.M., 840
Lee M.A., 541
Lee M.R.F., 644
Lenk I., 830
Lessire F., 181, 547
Lewandowski I., 429
Lewis E., 521, 556, 616, 628, 792
Liaudanskiene I., 187
Lind V., 563
Lindvall E., 498
Linsler D., 385
Litrico I., 112
Lloyd D.C., 855
Loges R., 125, 492
Loit E., 315
López-Sánchez A., 382
Louarn G., 112, 115, 122, 242
Lovatt A., 826
Lowe M., 350, 852, 867
Ludvíková V., 324
Ludwig B., 385
Ługowska M., 414
Lüscher A., 109, 166, 321, 722, 874
Lynch M.B., 677, 783, 789
Maass B.L., 160
MacAdam J.W., 884
Macfarlane A., 826
Macháč R., 502
Maczey N., 236
Makovníková J., 370
Maksimović N., 597
Mălinaş A., 298
Mandić V., 597
Marichal M. de J., 169
Marković, J., 291
Marley C.L., 267, 404, 651
Marshall A.H., 350, 855, 867
Martin B., 553
Martin C., 734
Martínez-Fernández A., 587, 590, 622
Maruşca T., 887
Matthews J.M., 309
McConnell D., 309
McConnell D.A., 541
McDonagh J., 836
McElhinney C., 610
McEniry J., 483
McEvoy M., 833, 836, 843
McHugh N., 843
Meek W.R., 254

Heavy metals in urban green cuttings

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Abstract

Urban grass cuttings are a potential source for energy production from biomass. However, concerns about heavy metal contamination, amongst others, have hampered the practical implementation in the past. At 10 sites within the city of Kassel, samples of roadside verge green cuttings were taken from a 2-cut and a 4-cut regime to analyse the heavy metal content (Cd, Cr, Cu, Mn, Pb, Zn). Elemental concentrations were measured with ICP-OES after microwave digestion. Cd and Pb could barely be detected. Cr contents were lower than in common agricultural grass and Cu as well as Zn contents were slightly higher. Mn contents meet the agricultural reference value. All elements fall below the limiting value of DIN EN 14961-6 for herbaceous biofuels (Mn is not considered by the DIN). Differences between 2- and 4-cut regimes were not statistically significant. Heavy metal contamination of municipal green cuttings is of little significance regarding energy recovery. Considering implementation opportunities of suitable technology is suggested.

Keywords: heavy metals, energy recovery, roadside verges, green cuttings

Introduction

Municipal grass cuttings are regularly mulched or composted, thereby stressing public budgets as well as the climate by the release of greenhouse gases. Utilization of green cuttings in energy recovery is possible with adapted technology but municipalities shy away from taking advantage of this opportunity because of concerns about the heavy metal content of the material and related legal uncertainties. Heavy metals occur naturally in soils, serving as micro-nutrients to organisms, but they can be enriched by human activity (e.g. traffic, industry) to potentially toxic levels (Cachada *et al.*, 2012). In the city of Kassel, grass cuttings (main grass species: *Lolium perenne* L. and *Festuca rubra* L.) from roadside verges were investigated for their heavy metal content (Cd, Cr, Cu, Mn, Pb, Zn). Grass cuttings are used for anaerobic digestion as well as for solid biofuel production (Prochnow *et al.*, 2009). As advancing stage of vegetation usually causes decreasing biogas yields due to an increasing content of crude fibre, we used a 4-cut management for estimating the biogas potential (Prochnow *et al.*, 2009). For the IFBB technology, which is adapted to fibre-rich material (Hensgen *et al.*, 2011), a 2-cut management is preferable due to economic and ecological reasons. In German legislation, there are no limiting values for heavy metals in material for energy recovery but there are limiting values regarding fuel specifications. Here, DIN EN 14961-6 is used as a reference, which specifies non-woody pellets for non-industrial use (DIN, 2012).

Materials and methods

In the city of Kassel, 10 dispersed sites were selected and divided into a 2-cut regime and a 4-cut regime with an area of 40 m^2 each. In 2013, samples were taken in triplicate for each site and cutting regime, and cut at 5 cm height using scissors. Cutting was done at 6-week intervals for the 4-cut regime and 12-week intervals for the 2-cut regime. Additionally, silage was produced from material from the 2-cut regimes. At a minimum of 6 weeks after the harvest date, the silage was processed with the IFBB-technique, with a tap water addition of ratio 4 to

1 and a mashing temperature of 40°C for 15 minutes. The material was dewatered by a screw press of stainless steel containing Cr (standard machinery configuration). Samples of the press cake (PC) and raw material were dried at 65°C. An open microwave digestion was conducted with 3.5 mL HNO_3 and 3.5 mL H_2O_2 added to 0.25 g of dried plant material. The mixture was allowed to react at room temperature overnight (minimum 12 hours) and placed in a microwave oven (1200 W, 0 psi), diluted until 25 mL, and filtered on an acid-resistant filter. The filtrate was analysed for trace elements with Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES: Varian vista MPX, Varian Palo Alto, USA). For statistical analysis Mann-Whitney U test was used, applying R (A Language and Environment for Statistical Computing, R Core TeamVienna, Austria, 2013).

Results and discussion

All of the investigated heavy metals (Cd, Cr, Cu, Mn, Pb and Zn) were detected in the samples. However, the contents of Cd and Pb were lower than the detection limits (0.4/4 mg kg^{-1} DM, respectively) in 99% of samples and therefore they fall below the limiting values of DIN EN 14961-6 and were close to values found by other authors in uncontaminated grass (Cd: 0.27 mg kg^{-1} DM, Pb: 3.3 mg kg^{-1} DM (Kabata-Pendias, 2011)). Mean Cr concentrations were 0.66±0.18 mg kg^{-1} DM in the 2-cut regime and 0.67±0.09 mg kg^{-1} DM in the 4-cut regime (Figure 1).
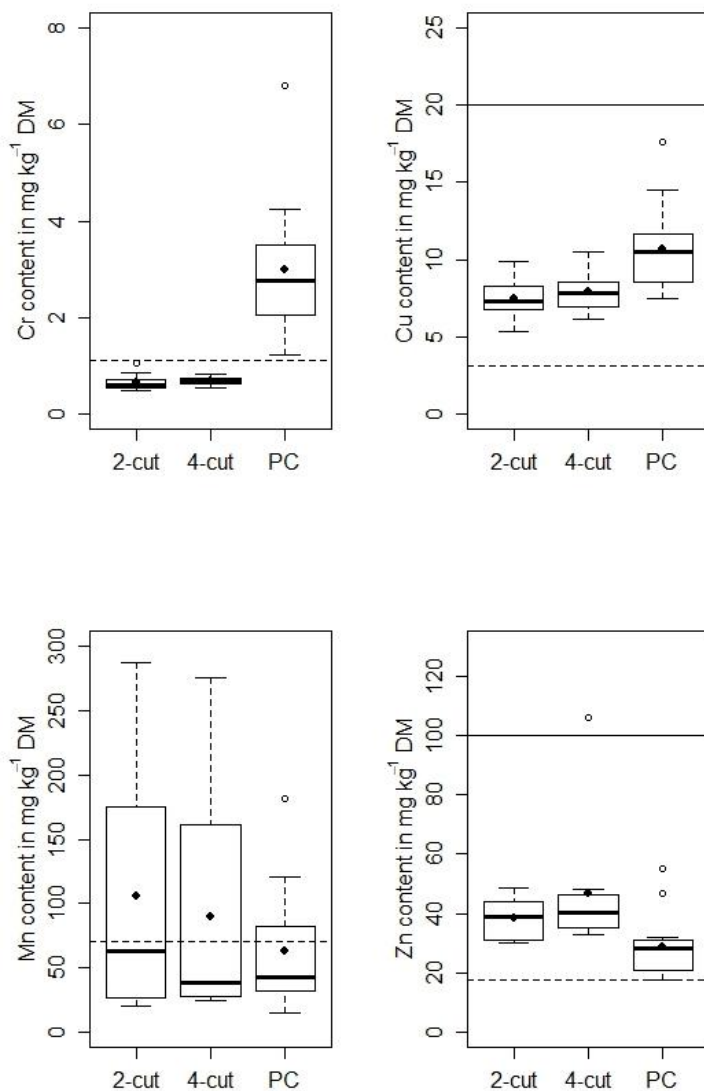

Figure 1. Contents of Cr, Cu, Mn and Zn in mg kg^{-1} DM with median (solid line in box) and arithmetic mean (full dot). Broken lines indicate element contents of agricultural grass (Kabata-Pendias, 2011; Lindström *et al.*, 2013) and solid lines indicate DIN limiting values (not shown for Cr (limiting value: 50 mg kg^{-1} DM) and Mn (not considered by the DIN)). In 53 of 175 samples, the Cr content was below the detection limit and was therefore not considered in statistical analysis.

Both were below the contents found in common grass of 1.1 mg kg^{-1} DM (Kabata-Pendias, 2011) and about 100 times lower than the DIN limiting value of 50 mg kg^{-1} DM. The Cr content in the PC was higher (3±1.37 mg kg^{-1} DM) than the usual content of non-urban grasses but still below the DIN value. Similarly, concentrations of Cu and Zn in municipal grass samples of both cutting regimes, and of the PC, exceeded contents of uncontaminated grass. The concentrations of Cu were 7.45±1.31, 7.95±1.31 and 10.67±2.79 mg kg^{-1} DM in the 2-cut and 4-cut regime and the PC, respectively, compared to a common value of uncontaminated grass of 3.1 mg kg^{-1} DM (Lindström *et al.*, 2013). Zn contents of 38.26±7.34, 46.59±21.54 and 28.65±10.31 mg kg^{-1} DM were determined in the 2-cut and 4-cut regime and the PC, respectively, while a natural content 17.7 mg kg^{-1} DM can be assumed (Lindström *et al.*, 2013). Nevertheless, concentrations of Cu and Zn fall below the DIN limiting values of 20 and 100 mg kg^{-1} DM, respectively. Concentrations of Mn in the raw material are in the range of common agricultural grass, with the medians below and the means of 106.05±104.71 and 89.82±90.47 mg kg^{-1} DM in the 2-cut and 4-cut regime, respectively, above the value of 70 mg kg^{-1} DM (Kabata-Pendias, 2011). In the PC both values, median and mean (62.83±47.2 mg kg^{-1} DM), fall below this value. No significant differences in heavy metal concentrations of the treatments were found. Low contents of heavy metals in roadside material have also been observed for Cu, Pb and Zn in Italian ryegrass (*Lolium multiflorum* Lam.) in Finland (Yläranta, 1994). However, uptake of heavy metals is highly dependent on plant species and soil conditions.

Conclusion

The heavy metal content (Cd, Cr, Cu, Mn, Pb, Zn) of green cuttings from roadside grass in the city of Kassel is low, in general. Even considering a certain amount of contamination from the machinery, the contents of the investigated elements fall below the DIN limiting values. The results therefore provide no reason for not utilising the material in energy recovery systems. Thus, it is suggested that the economic and ecological conditions, which are necessary to successfully maintain green areas for energy recovery purposes, should be explored.

Acknowledgements

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