



**HAL**  
open science

## Potential of legume-based grassland-livestock systems in Europe

Andreas Lüscher, Irene Mueller-Harvey, Jean-François Soussana, R. M. Rees, Jean-Louis Peyraud

► **To cite this version:**

Andreas Lüscher, Irene Mueller-Harvey, Jean-François Soussana, R. M. Rees, Jean-Louis Peyraud. Potential of legume-based grassland-livestock systems in Europe. 17th Symposium of the European Grassland Federation (EGF), Jun 2013, Akureyri, Iceland. European Grassland Federation, Grassland Science in Europe, 18, 2013, Grassland Science in Europe. hal-01210591

**HAL Id: hal-01210591**

**<https://hal.science/hal-01210591>**

Submitted on 3 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Potential of legume-based grassland-livestock systems in Europe

Lüscher A.<sup>1</sup>, Mueller-Harvey I.<sup>2</sup>, Soussana J.F.<sup>3</sup>, Rees R.M.<sup>4</sup> and Peyraud J.L.<sup>5</sup>

<sup>1</sup> *Agroscope Reckenholz-Tänikon Research Station ART, 8046 Zurich, Switzerland*

<sup>2</sup> *University of Reading, Reading RG6 6AT, United Kingdom*

<sup>3</sup> *INRA, Grassland Ecosystem Research, 63100 Clermont-Ferrand, France*

<sup>4</sup> *Scotland's Rural College, Edinburgh EH9 3JG, United Kingdom*

<sup>5</sup> *INRA, UMR PEGASE, 35590 St Gilles, France*

Corresponding author: andreas.luescher@art.admin.ch

## Abstract

European grassland-based livestock production systems are challenged to produce more milk and meat to meet increasing world demand and to achieve this by using fewer resources. Legumes offer great potential for coping with such requests. They have numerous features that can act together at different stages in the soil-plant-animal-atmosphere system and these are most effective in mixed swards with a legume abundance of 30-50%. The resulting benefits are a reduced dependency on fossil energy and industrial N fertilizer, lower quantities of harmful emissions to the environment (greenhouse gases and nitrate), lower production costs, higher productivity and increased protein self-sufficiency. Some legume species offer opportunities for improving animal health with less medication due to bioactive secondary metabolites. In addition, legumes may offer an option for adapting to higher atmospheric CO<sub>2</sub> concentrations and to climate change. Legumes generate these benefits at the level of the managed land area unit and also at the level of the final product unit. However, legumes suffer from some limitations, and suggestions are made for future research in order to exploit more fully the opportunities that legumes can offer. In conclusion, the development of legume-based grassland-livestock systems undoubtedly constitutes one of the pillars for more sustainable and competitive ruminant production systems, and it can only be expected that legumes will become more important in the future.

Keywords: yield, symbiotic N<sub>2</sub> fixation, forage quality, nutritive value, voluntary intake, animal performance, greenhouse gas emission, nitrate leaching, condensed tannins, animal health, clover, climate change, energy, plant secondary metabolites, management

## Introduction

European grassland-based livestock production systems have changed considerably over the last two decades and will continue to evolve in response to societal and environmental pressures. Grassland production will need to keep pace with requirements for higher meat and milk production from ruminant systems and with a changing climate. At the same time, grassland production needs to minimize competition from arable land and between food and feed production, and preserve biodiversity and ecosystem services (Thornton, 2010). Legumes offer important opportunities for sustainable grassland-based animal production because they can contribute to important key challenges by (i) increasing forage yield, (ii) substituting inorganic N fertilizer inputs with symbiotic N<sub>2</sub> fixation, (iii) mitigating and supporting adaptation to climate change, as elevated atmospheric CO<sub>2</sub>, warmer temperatures and drought-stress periods increase, and (iv) increasing the nutritive value of herbage and raising the efficiency of conversion of herbage to animal protein.

A concerted programme of research supported by the European Commission is devoted to improving our understanding of the roles played by legumes in grassland systems. Of

particular interest are four current initiatives: MultiSward ([www.multisward.eu](http://www.multisward.eu)), Legume Futures ([www.legumefutures.eu](http://www.legumefutures.eu)), AnimalChange ([www.animalchange.eu](http://www.animalchange.eu)) and LegumePlus ([www.legumeplus.eu](http://www.legumeplus.eu)). The objective of MultiSward is to support developments and innovations for grassland production and management in different European farming systems, and under different pedoclimatic and socio-economic conditions. They focus (i) on enhancing the role of grasslands at farm and landscape levels in terms of environmental services and biodiversity, and (ii) on optimizing their economic, agronomic and nutritional contributions for innovative and sustainable ruminant production systems. An important aspect is the investigation of the effects of multispecies mixtures, where grasses and forbs are combined with shallow and deep rooting clovers, on forage production, grazing systems and ecosystem services. The Legume Futures project is designed to optimize the use of legumes in European agriculture. It takes account of the agronomic, economic and environmental impacts of legume cultivation and uses experimentation and modelling to develop novel legume-based cropping systems. AnimalChange will provide options for the livestock sector to cope with climate change in the future: (i) by reducing uncertainties concerning greenhouse gas (GHG) emissions from livestock systems, (ii) by developing cutting-edge technologies for mitigation and adaptation to climate change, (iii) by assessing the vulnerability of livestock to climate change and feedbacks on GHG emissions, and (iv) by providing direct support to set up policies for the livestock sector in order to mitigate and adapt to climate change. Legumes can offer important options and these will be examined in this project. The key objective of LegumePlus is to investigate how bioactive forage legumes, in particular sainfoin (*Onobrychis viciifolia* Scop.) and birdsfoot trefoil (*Lotus corniculatus* L.), can improve protein utilization in ruminant livestock farming. Of equal importance is the potential that these legumes offer for combatting parasitic nematodes in ruminants. This project will study the combined effects of nitrogen and methane emissions plus their impact on food quality, e.g. milk, cheese and meat quality.

This paper, by authors involved in these four European research programmes, aims to review the literature for opportunities that forage legumes can offer in order to meet key challenges, which will be faced by future grassland-animal husbandry systems. The authors also seek to highlight research that is needed to enable increased utilization of legumes in Europe. It is, however, not the aim to give detailed profiles of individual forage legume species as this information was compiled by Frame (2005) for more than 30 legume species.

### **Political and socio-economic background**

Over many years, the European Union's Common Agricultural Policy (CAP) has encouraged large increases in agricultural production and the intensification of agricultural systems. In addition, low energy prices during the second half of the 20<sup>th</sup> century resulted in an abundant supply of cheap synthetic nitrogen (N) fertilizer, which further reduced the demand for production from legume-based grasslands (Rochon *et al.*, 2004). These changes have had adverse environmental impacts by increasing greenhouse gas emissions and by lowering biodiversity, which has been linked strongly to the use of synthetic N fertilizer (Schulze *et al.*, 2009; Stoate *et al.*, 2009). There is no specific requirement under current EU agricultural policy to support legume-based cropping systems or to develop home-grown protein crops, but revisions to the policy are envisaged by the European Commission by 2020, which are likely to encourage synergies between crop and livestock farming in order to make better use of protein sources (European Commission, 2010).

World-trade agreements have promoted imports of grain legumes into Europe and have led to lower European production despite increased consumption. This dependence of Europe's livestock industry on imports of grain legumes has raised questions about the sustainability and security of such a production model against a background of increasing demands for food

and concerns about the environmental impact of livestock production systems (Godfray *et al.*, 2010). Galloway *et al.* (2008) estimated that South America exported a net amount of 2.3 Mt of N in grain legumes to Europe in 2004. As well as creating an imbalance in global N cycles, this export of grain legumes to Europe has also led to a large-scale change in land use in South America, as forests have been cleared for soya production (Weightman *et al.*, 2011). Governments of European countries are becoming increasingly concerned about the security of their protein supplies, and the UK, Germany and the European Parliament have recently discussed the development of new policies to support national protein security (Aigner, 2009; European Parliament, 2011).

The magnitude of benefits offered by the use of legumes in European farming systems is informed by scientific studies in a number of areas. This paper summarizes some of the key issues, and highlights remaining areas of uncertainty. Such uncertainties, however, need not act as a barrier to developing policy where the balance of evidence supports change. The large amounts of public funding (in the Common Agricultural Policy) that are used to support European Agriculture can be expected to deliver public benefits (such as those associated with reduced environmental impact) as well as a sustainable food production sector.

### **Legume-grass swards a key to increased yield**

Under fertile agricultural conditions monocultures of selected, highly productive grass species give high forage yields with high inputs of fertilizer nitrogen (N) (Frame, 1991; Daepf *et al.*, 2001). The need to increase not only productivity but also resource efficiency (sustainable intensification) poses new challenges for agriculture. Plant communities with higher species number (richness) are expected to (i) utilize available resources better due to species niche complementarity, (ii) have a higher probability of showing positive interspecific interactions, and (iii) may contain highly productive species that dominate the community (selection effect) (Tilman, 1999; Loreau and Hector, 2001; Loreau *et al.*, 2001). Thus, cropping mixtures could be a promising strategy for sustainable intensification.

Indeed, many experiments in nutrient-poor grasslands have shown that biomass production was enhanced in species-rich swards, compared with the average monoculture yield (Spehn *et al.*, 2002; Hille Ris Lambers *et al.*, 2004; Hooper and Dukes, 2004; Hooper *et al.*, 2005; Roscher *et al.*, 2005; Marquard *et al.*, 2009; Mommer *et al.*, 2010). In a meta-analysis of 44 biodiversity experiments that manipulated plant species richness, Cardinale *et al.* (2007) found that the most diverse mixtures, on average, achieved a yield benefit of +77% compared to the average monoculture. However, compared to the most productive monoculture, these mixtures showed a yield disadvantage of -12%. Transgressive overyielding (mixtures outperform the best monoculture; Trenbath, 1974; Schmid *et al.*, 2008) occurred in only 12% of the experiments and it took, on average, about five years to become evident. However, in an agronomic context, mixtures with transgressive overyielding are clearly preferred, as stakeholders can select the highest yielding species for monoculture cultivation, and any mixture performance has to compete against this high benchmark.

A pan-European experiment carried out across 31 sites in 17 countries under the auspices of COST Action 852 'Quality legume-based forage systems for contrasting environments' ([www.cost.eu/domains\\_actions/fa/Actions/852](http://www.cost.eu/domains_actions/fa/Actions/852)) tested whether higher yields, compared with monocultures, can be achieved under typical agricultural conditions with grass-legume mixtures containing four species (Kirwan *et al.*, 2007; Lüscher *et al.*, 2008; Nyfeler *et al.*, 2009). These four species represented four functional groups of plants: a fast-establishing grass, a fast-establishing legume, a slow-establishing grass and a slow-establishing legume. These functional groups of plant species were chosen to maximize beneficial interspecific interactions: legumes enable symbiotic fixation of atmospheric nitrogen, and fast/slow combinations were intended to maximize sward cover by species with known different

temporal patterns of development. The legume species examined were *Trifolium pratense* L. (red clover, 29 sites), *Trifolium repens* L. (white clover, 26), *Medicago sativa* L. (lucerne, 3), *Medicago polymorpha* L. (burr medic, 2), and *Trifolium ambiguum* M. Bieb. (Caucasian clover, 2) (Finn *et al.*, 2013).

Across the three years, the yield of sown species (total yield excluding weed biomass) was higher in the mixture than the average monoculture yield for 99.7% of the mixture plots, with a yield advantage of 77% of the mixtures above the average monoculture. It was most remarkable that transgressive overyielding was achieved in 79% of the mixture plots and the yield advantage of the average mixture was 18%, compared with the highest yielding monoculture (Finn *et al.*, 2013). At the Swiss site, which tested red and white clover, a comparison across N fertilizer input levels revealed a high potential for N fertilizer replacement: grass-clover mixtures containing 40-60% clover and receiving 50 or 150 kg ha<sup>-1</sup> yr<sup>-1</sup> fertilizer N achieved the same yield as grass monocultures fertilized with 450 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Nyfeler *et al.*, 2009). Over the whole pan-European experiment advantages of grass-legume mixtures were surprisingly robust: they persisted over the three experimental years, over the different legume species tested, and over the large climatic gradient covered by the experimental sites, spanning a latitudinal range from 40°44'N (Sardinia, Italy) to 69°40'N (Tromsø, Norway) (Finn *et al.*, 2013; Sturludóttir *et al.*, 2013).

Evenness, the degree of even species abundance in swards, had a highly significant effect on the yield and diversity effect (excess of mixture performance over that expected from average monoculture performances) (Kirwan *et al.*, 2007; Finn *et al.*, 2013; Sturludóttir *et al.*, 2013). Increase in mixture evenness at low values resulted in a steep increase of the diversity effect (Connolly *et al.*, 2013). However, these benefits of raising evenness showed a fast saturation and the diversity effect remained relatively constant across a wide range of medium to high evenness values (Kirwan *et al.*, 2007; Connolly *et al.*, 2013; Finn *et al.*, 2013; Sturludóttir *et al.*, 2013), indicating a high robustness of the diversity effect to changes in relative abundance of different species. Because high evenness in these mixtures was jointly linked to legume percentages of 35-65%, results of Finn *et al.* (2013) are in agreement with the recent finding of significant transgressive overyielding in mixtures over a wide range of about 30 to 80% clover percentage in the sward (Nyfeler *et al.*, 2009).

These findings suggest that grass-legume mixtures offer a great potential for increased production even at relatively low species richness. In the pan-European experiment of Finn *et al.* (2013), grass-legume mixtures outperformed both grass and legume monocultures. Because symbiotic N<sub>2</sub> fixation cannot explain the highly significant yield advantage of mixtures over legume monocultures (detailed results in Nyfeler *et al.*, 2009), access to atmospheric N<sub>2</sub> could not have been the only factor causing increased mixture yields. In diversity experiments, the positive interactions between N<sub>2</sub>-fixing legumes and non-N<sub>2</sub>-fixing plant species often contributed to a significantly larger extent to mixing effects in biomass yield than the interactions between other functional groups (Spehn *et al.*, 2002; Li *et al.*, 2007; Temperton *et al.*, 2007; Kirwan *et al.*, 2009; Nyfeler *et al.*, 2009). However, other trait combinations can yield important diversity effects also (Van Ruijven and Berendse, 2003; Roscher *et al.*, 2008). In the pan-European experiment, mixtures strongly benefitted from the combination of fast-establishing with slow-developing, but temporally persistent species (Kirwan *et al.*, 2007; Finn *et al.*, 2013). Further research is needed to quantify the mechanisms of the complementarity for a variety of functional traits and their contributions to the effects on mixture yield. This knowledge would allow the design of mixtures that combine species with high complementarity of different traits, which in turn will result in optimized resource exploitation through niche differentiation (Hill, 1990; Lüscher and Jacquard, 1991). Such research should also include legumes other than *Trifolium pratense* and *T. repens*, on which most evidence is based so far. In particular, legumes known to perform well under cold

and/or dry conditions should be tested. Extension of designs will also reveal whether a further increase in species richness can lead to an additional gain in diversity effects and mixture yields (Connolly *et al.*, 2013).

### **Legume yield is a main driver for large N inputs by symbiotic N<sub>2</sub> fixation**

The massive acceleration of the global N cycle by N fertilizer from the industrial Haber-Bosch process and by N emissions from the combustion of fossil fuels, has enabled humankind to increase greatly food production. This, however, has also led to a host of environmental problems, ranging from eutrophication of terrestrial and aquatic ecosystems to global acidification and climate change (Gruber and Galloway, 2008; Rockström *et al.*, 2009; Vörösmarty *et al.*, 2010; Hooper *et al.*, 2012). Anthropogenic N released to the environment is greater than 160 teragrams (Tg) N yr<sup>-1</sup>, which clearly exceeds the amount supplied by biological N<sub>2</sub> fixation in natural systems (110 Tg N yr<sup>-1</sup>) (Gruber and Galloway, 2008; Herridge *et al.*, 2008). Substitution of industrial N fertilizer by improved exploitation of N<sub>2</sub> fixation from symbiosis of legumes with *Rhizobium* bacteria would thus be an important contribution to environmental-friendly and resource-efficient agricultural systems.

In grasslands, symbiotically fixed N<sub>2</sub> by legumes can range from 100 to 380 kg of N ha<sup>-1</sup> yr<sup>-1</sup> and exceptionally large amounts of > 500 kg of N ha<sup>-1</sup> yr<sup>-1</sup> have also been reported (Boller and Nösberger, 1987; Ledgard and Steele, 1992; Zanetti *et al.*, 1997; Carlsson and Huss-Danell, 2003). In mixed grass-legume systems, additional amounts of 10-75 kg of N ha<sup>-1</sup> yr<sup>-1</sup> are transferred from legumes to grasses, while the amount transferred depends on the donor and the receiver plant species (Pirhofer-Walzl *et al.*, 2012). The control of symbiotic N<sub>2</sub> fixation operates through a series of ecophysiological triggers (Hartwig, 1998; Soussana *et al.*, 2002), with the amount of symbiotically fixed N<sub>2</sub> being tightly coupled to the gap between N demand (sink) and N availability (source) from mineral-N sources at several scales from plant physiology to the whole ecosystem (Soussana and Hartwig, 1996; Hartwig, 1998; Soussana and Tallec, 2010).

In grass-clover mixtures containing red and white clover, Nyfeler *et al.* (2011) observed stimulatory effects of the accompanying grasses on the symbiotic N<sub>2</sub> fixation activity of clover (% N derived from symbiosis). This effect was so strong that: (i) the amount of N from symbiosis was maximized not in pure clover stands but in mixtures with 60-80% of clovers, and (ii) 40-60% of clovers in the mixture were sufficient to attain the same amount of N from symbiosis as in pure clover stands. This stimulatory effect fits well into the sink/source model of the regulation of symbiotic N<sub>2</sub> fixation. The activity of symbiotic N<sub>2</sub> fixation of clover plants was very high in grass-dominated swards, where the availability of mineral N to clovers was very low; this was evident from the fact that most of the N from mineral sources was taken up by the competitive grass component (Nyfeler *et al.*, 2011). However, in clover-dominated swards (> 60% of clovers), the activity of symbiotic N<sub>2</sub> fixation was down-regulated. This was due to the clovers' adequate access to mineral N sources because of low grass abundance (Nyfeler *et al.*, 2011) as well as due to a significantly reduced N demand of the whole sward in clover-dominated stands, as these were less productive than well balanced mixtures (Nyfeler *et al.*, 2009). The sensitivity of legume plants to down-regulate their symbiotic N<sub>2</sub> fixation (percentage of N derived from symbiosis) seems to show interspecific differences (Rasmussen *et al.*, 2012). In general, forage legumes grown in mixtures (with a reasonable abundance of grasses) receive most of their N (> 80%) from symbiotic N<sub>2</sub> fixation (Boller and Nösberger, 1987; Heichel and Henjum, 1991; Carlsson *et al.*, 2009; Oberson *et al.*, 2013), which implies that the amount of N derived from symbiosis depends on the forage legume dry matter production (Unkovich *et al.*, 2010; Lüscher *et al.*, 2011).

Legumes have often been studied in intensively managed grasslands under productive soil and climatic conditions. In extensively managed non- or low-fertilized grassland experiments,

high values of the proportion of N derived from symbiosis have also been measured in a range of legume species (Carlsson *et al.*, 2009; Roscher *et al.*, 2011). However, low values of the amount of N derived from symbiotic N<sub>2</sub> fixation were observed at low temperatures in two growth chamber experiments on nutrient solution (Kessler *et al.*, 1990; Nesheim and Boller, 1991). This was not only related to low growth and total nitrogen accumulation of white clover, but also to a marked reduction of the proportion of N derived from symbioses. Thus, the authors concluded that the negative effect of low temperature on processes of nitrogen fixation-nodulation (e.g., Roughley and Dart, 1970) and nitrogenase activity (e.g., Cralle and Heichel, 1982) were primarily responsible for the small contribution of nitrogen fixation to nitrogen nutrition of white clover at low temperatures. Only few field studies have been published on symbiotic N<sub>2</sub> fixation under marginal conditions at high altitude (e.g. Bowman *et al.*, 1996) or high latitude (e.g. Henry and Svoboda, 1986; Sparrow *et al.*, 1995). Jacot *et al.* (2000a; 2000b) studied the significance of symbiotic N<sub>2</sub> fixation for the legumes and for the N balance of the whole grassland ecosystem of species-rich semi-natural pastures in the Alps. The legume species examined were *Lotus corniculatus* L., *L. alpinus* Schleicher, *Vicia sativa* L., *Trifolium pratense* L., *T. repens* L., *T. nivale* Sieber, *T. thalii* Vil., *T. badius* Schreber, and *T. alpinum* L.. Along an altitudinal gradient from 900 m a.s.l. to the altitudinal limit of legume occurrence at 2600 m a.s.l., all the legume species met most of their N requirements from symbiosis (59 to 90%). This suggests that symbiotic N<sub>2</sub> fixation is well adapted both to the climatic and acidic soil conditions (pH 5.6 to 4.1) of the sites investigated. Nevertheless, the amount of N derived from symbiosis decreased significantly with increasing altitude (18 to 1 kg N ha<sup>-1</sup> yr<sup>-1</sup>) due to a strong decrease in the total productivity of the plant community and to a decrease from 15 to 4% of legumes in the sward.

These findings and the sink/source model of regulation of symbiotic N<sub>2</sub> fixation at the ecosystem level have several critical implications for the exploitation of atmospheric N<sub>2</sub> in grassland systems. First, grass-clover mixtures can potentially fix more N<sub>2</sub> from the atmosphere than clover monocultures. Second, there is no trade-off between high productivity and high gains of symbiotically fixed N<sub>2</sub> because they are positively linked through N demand (sink). Consequently, sufficient availability of other nutrients, such as P, K, and S, is crucial for stimulating demand of N from symbiosis (Hartwig, 1998; Brown *et al.*, 2000; Tallec *et al.*, 2009). Third, very high inputs of N into the ecosystem through symbiosis can cause a risk for N losses into the environment. However, such unwanted N losses can be prevented by sufficiently great abundance of grasses in the sward to ensure a highly competitive uptake of mineral N from the soil.

## **Efficiency of conversion of forage into animal products**

### *Legumes increase nutritive value and voluntary intake*

Livestock production is influenced by both the nutritive value and the voluntary intake of forages. The chemical composition and nutritive value of forages have been summarized by INRA (2007). Compared to perennial ryegrass (*Lolium perenne* L.), white clover, red clover and lucerne have high concentrations of crude protein (CP) and minerals, such as calcium, but contain relatively low concentrations of water-soluble carbohydrates (WSC). The nutritional advantage of white clover over grasses is well established (Beever *et al.*, 1986; Peyraud, 1993). Organic matter digestibility and net energy concentration, as well as the supply of metabolizable protein, are generally higher for white clover than for grasses (INRA, 2007). These results reflect a lower proportion of structural cell wall components, which are less digestible than cell contents. Red clover and lucerne are less digestible and their net energy concentration is lower than that of white clover at a similar growth stage, the difference being greater for lucerne (5.54, 6.10 and 7.17 MJ kg DM<sup>-1</sup> for lucerne, red clover and white clover,

respectively (INRA, 2007)). These values are further reduced in silage and hay. Lucerne, and to a lesser extent red clover, should be cut at an early growth stage in order to maximize the net energy concentration of the conserved forage. In contrast, their net energy and metabolizable protein content is high when fed as fresh forage, and is almost at the recommended level for optimal feeding of dairy cows (INRA, 2007) and higher than that recommended for low-producing cows. Hay-making maintains the high metabolizable protein content but ensiling reduces its content.

Voluntary intake of dry matter (DM) of legumes is 10 to 15% greater than that of grasses of similar digestibility and this is true whether forage legumes are fed as silage, hay or fresh (INRA, 2007). These differences are attributed to a lower resistance of legumes to chewing, a faster rate of digestion and a faster rate of particle breakdown and clearance from the rumen (Waghorn *et al.*, 1989; Jamot and Grenet, 1991; Steg *et al.*, 1994; Dewhurst *et al.*, 2009), which in turn reduce rumen fill. Dewhurst *et al.* (2003) reported that DM intake of silage is increased by 2 to 3 kg when cows are fed red clover or white clover silages, compared with perennial ryegrass silage. White clover is often used in a mixture with perennial ryegrass and this raises the question of the optimal proportion of white clover. Harris *et al.* (1998) showed that the DM intake of housed dairy cows was at its maximum when white clover reached 60% in the forage.

Herbage intake by grazing livestock is generally constrained by herbage allowance or pasture structure. At the same herbage allowance, Alder and Minson (1963) found that herbage intake was 15 to 20% higher with pure lucerne relative to pure cocksfoot pastures. The beneficial effects of white clover on herbage intake and performance by livestock grazing a white clover-grass pasture have also been demonstrated (Wilkins *et al.*, 1994; Ribeiro-Filho *et al.*, 2003; 2005). The difference in daily herbage intake increased with increasing percentage of clover in the diet and reached 1.5 kg on average in these latter studies. In addition to the positive effect of legumes on voluntary intake, it is also probable that leaves of legumes are more favourable for intake than stems and sheaths of grasses, particularly during the spring-heading period. Thus, Ribeiro-Filho *et al.* (2003) have reported a higher rate of intake on mixed white clover/perennial ryegrass pastures compared with pure perennial ryegrass pastures.

An additional benefit of white clover is that the rate of decline in nutritive value throughout the plant-ageing process is much lower than for grasses. This has been known for many years (Ulyatt, 1970). Digestibility and voluntary DM intake decreased by 20 g kg<sup>-1</sup> and 0.2 kg day<sup>-1</sup> per week during the first growth of grass herbage, whereas these decreases were half that for white clover herbage (INRA, 2007; Peyraud *et al.*, 2009). Peyraud (1993) and Delaby and Peccatte (2003) reported the digestibility of DM to be >75% after 7 weeks of regrowth or at the flowering stage during the first growth in spring. Ribeiro-Filho *et al.* (2003) showed that the DM intake of herbage declined by 2.0 kg day<sup>-1</sup> on a predominantly grass pasture compared to 0.8 kg day<sup>-1</sup> on mixed pastures. This makes mixed pastures much easier to manage than pure grass pastures for maintaining their nutritive value and makes mixed pastures particularly attractive on farm because of their greater flexibility. For example, it allows intervals between two successive grazing periods of more than 4 to 6 weeks in the summer. For lucerne and red clover, the decline in nutritive value with advancing maturity is intermediate between that of white clover and grasses (INRA, 2007). Sturludóttir *et al.* (2013) observed that the yield increase of legume-grass mixtures compared to monocultures was not accompanied by a reduction in herbage digestibility and crude protein concentration that is usually observed with increased DM yield. Low-lignin lucerne cultivars could be another opportunity to further reduce the decline of nutritive value with advancing maturity (Undersander *et al.*, 2009).



### *Legumes increase performance of livestock*

Several experiments have shown that pure legume silages and legume-dominated silages can increase milk production compared to pure grass silages (Castle *et al.*, 1983; Dewhurst *et al.*, 2003). Chenais (1993) summarized the results of ten French experiments, which had studied the effect of a mixed diet, based on maize (*Zea mays* L.) silage and red clover, or lucerne silages compared with pure maize silage-based diets. The mixed diets led to similar levels of performance by dairy cows when the legume silages were of a high nutritive value and in particular when their DM content was  $>300$  g kg<sup>-1</sup>. The same applied to beef production, where red clover silage made it possible to obtain growth rates that were identical to maize silage as long as it was well-preserved (Weiss and Raymond, 1993). However, it should be pointed out that legumes can be difficult to conserve; special care must be taken to ensure good silage quality and to minimize leaf losses during hay-making (Arnaud *et al.*, 1993). Increasing the concentration of total non-structural carbohydrates (TNC) of legumes will undoubtedly facilitate the production of high-quality silages and increase animal performance. This can be achieved by cutting the plot during the afternoon when sugar content raised its maximum (Brito *et al.*, 2008; Pelletier *et al.*, 2010; Morin *et al.*, 2011). Plant breeding and gene manipulation might also be an option to increase TNC in legume plants (Tremblay *et al.*, 2011).

A higher content of white clover in the pasture increased the daily milk yield of cows by 1 to 3 kg in several short-term experiments when the same DM herbage allowance was offered to dairy cows grazing pure perennial ryegrass pasture and mixed pastures (Philips and James, 1998; Ribeiro-Filho *et al.*, 2003). In a study with housed dairy cows, milk yield increased with increasing white clover content in the diet and reached a maximum when the percentage of white clover averaged 50 to 60% (Harris *et al.*, 1998). Conversely, milk yield is reduced when the abundance of clover is low ( $<20\%$ , Gately 1981). As a consequence of higher energy intake, milk protein concentration tends to increase on mixed pastures. However, growth rates of growing cattle are relatively similar with these types of pasture. Nevertheless, on set-stocked swards, which were maintained at a similar height, mixed grass-legume pastures supported a slightly higher growth rate of lambs than fertilized grass pastures (Orr *et al.*, 1990; Speijers *et al.*, 2004).

As mixed pastures are managed with very low N fertilization, the biomass ha<sup>-1</sup> might be lower than from highly fertilized grass pastures at a same age of regrowth. Therefore, mixed pastures often have lower milk yields and liveweight gains ha<sup>-1</sup> than pure perennial ryegrass pastures as stocking rates are generally slightly lower in order to maintain similar herbage allowances (Institut de l'Elevage, 2004; Humphreys *et al.*, 2009). Difficulties in maintaining well balanced grass-legume mixtures and their tendency to lose key species (Guckert and Hay, 2001) may also be a reason for the preference of pure grass swards by farmers. Alternatively, good flexibility in utilization of mixed pastures allows the use of intervals between two successive grazings of more than 4-6 weeks in summer, thereby compensating for lower productivity without penalizing the performances of the cows.

### *Legumes show a low efficiency of N digestion in the rumen*

Losses of ruminal N in legume-fed ruminants are always high due to an imbalance between degradable N and fermentable energy in the forage. The rumen degradability of protein is higher for forage legumes in comparison with perennial ryegrass (Beever *et al.*, 1986). This leads to an inefficient utilization of forage N in the rumen and high urinary-N excretion (Peyraud, 1993). White clover increases N excretion relative to perennial ryegrass from 20.1 to 29.8 g kg<sup>-1</sup> DM intake and the amount of N that enters the duodenum is always below N intake, averaging 75% of N intake for white clover compared to 93% for ryegrass. From the

data of Ribeiro-Filho *et al.* (2005) it can be calculated that N excretion increased from 17.0 to 20.7 g kg<sup>-1</sup> milk on mixed white clover-perennial ryegrass pastures compared with perennial ryegrass pastures.

The WSC contained within the forage should be such as to balance the crude protein (CP) concentration of herbage in order to maximize microbial protein synthesis. However, the WSC content in temperate swards is variable and normally low. Promising results have been obtained by plant breeding and gene manipulation to increase WSC concentration in perennial ryegrass (Miller *et al.*, 2001) and this led to a slight increase in digestibility and a reduction in urinary-N losses (Miller *et al.*, 2001; Lee *et al.*, 2002). However, legume forages typically have high CP and low WSC concentrations. Increasing WSC concentrations might improve ruminal-N utilization and plant digestibility in the case of lucerne and red clover. Combining grasses with high WSC concentrations and legumes with low CP concentrations in mixed swards should allow a reduction in N fertilizer inputs and a reduction in the risk of high N excretion from livestock grazing swards with high CP concentrations. Significant variation within white clover and associated materials for lower CP and higher WSC concentrations has been identified (N.D. Scolan, pers. comm.). An experiment using mixed pastures based on perennial ryegrass with enhanced levels of WSC and white clover with variation in CP concentrations is being undertaken in the Multisward project (<http://www.multisward.eu>).

The extensive degradation of CP, which occurs during ensilage, worsens the imbalance between degradable protein and energy in legume silages and leads to inefficient N utilization and high urinary N excretion (Dewhurst *et al.*, 2003; Cohen *et al.*, 2006; Dewhurst *et al.*, 2009). Supplementation with cereal grains can overcome the relatively low energy concentration of legume silages and, hence, reduce urinary N losses per unit of forage intake (Cohen *et al.*, 2006). Legume silages or hays complement maize silage in mixed diets well (Chenais, 1993; Rouillé *et al.*, 2010) as they can provide sources of both degradable and undegradable protein. They also offer some potential to substitute imported soybean meal with home-grown protein, which will contribute towards protein self-sufficiency of livestock production on farm (Peyraud *et al.*, 2012).

### **Plant secondary metabolites are a key feature of legumes**

This section of the paper will focus on a few legumes that possess additional features which offer promise for ruminant nutrition and health, and for reducing greenhouse gas emissions. These features include tannins, polyphenol oxidase and protease enzymes (Mueller-Harvey, 2006; Kingston-Smith *et al.*, 2010). Table 1 lists key (dis)advantages of some current legume species with such features and are being investigated in the current EU LegumePlus project (<http://legumeplus.eu>). Sainfoin (*Onobrychis viciifolia* Scop.) holds particular promise for alkaline and drought-prone soils, which cover much of central and southern Europe (Sölter *et al.*, 2007). All legumes improve soil fertility and thus contribute to sustainability; but sainfoin contributes 16,200 kg of dry matter from fine roots ha<sup>-1</sup> compared to 4,200 kg ha<sup>-1</sup> from lucerne (Sergeeva, 1955). Together these legume species cover soil pH from 4 to 8.5 and temperature tolerance from southern to northern Europe. Red clover varieties have been improved for over 50 years, but few sainfoin or birdsfoot trefoil (*Lotus corniculatus* L.) cultivars are available. Plant breeding goals and achievements were recently reviewed for the main legume species red clover (Boller *et al.*, 2010), white clover (Abberton and Marshall, 2010) and lucerne (Veronesi *et al.*, 2010) but also for minor legume species (Piano and Pecetti, 2010) including birdsfoot trefoil and sainfoin.

Condensed tannins are oligomers and polymers of flavanols and have been found in a few forage legumes, such as birdsfoot trefoil, sainfoin, sulla (*Hedysarum coronarium* L.) and the flowers of *Trifolium* species (Waghorn *et al.*, 1998; Waghorn, 2008). Total concentrations and compositions depend on accession/variety (Häring *et al.*, 2007; Azuhwi *et al.*, 2011;

Stringano *et al.*, 2012), season (Theodoridou *et al.*, 2011), plant organ (Håring *et al.*, 2007) and processing method (Hoste *et al.*, 2006). Varieties with relatively stable concentrations and compositions will be needed to ensure that farmers can obtain reliable benefits from legumes containing condensed tannins. Whilst the biosynthesis of monomeric polyphenols and flavanols is now known, the search is still on for the genes and enzymes involved in the synthesis of condensed tannins (Dixon *et al.*, 2012). Although it is likely that the quantitative and qualitative traits (i.e. concentrations and structures) of condensed tannins are under genetic control (Scioneaux *et al.*, 2011), the question remains how amenable these traits are to improvement by plant breeding. Research will also be needed in order to develop screening tools that are suitable for breeding new legume varieties with optimized composition of condensed tannins.

Table 1. Advantages and disadvantages of bioactive legumes, which are suitable for covering a range of European environments and soil conditions.

Characteristics	Legume species		
	<i>Trifolium pratense</i> Red clover	<i>Lotus corniculatus</i> Birdsfoot trefoil	<i>Onobrychis viciifolia</i> Sainfoin
EU latitudes	35° to 64°	35° to 56°	35° to 54°
Soil pH:			
Tolerance	4.5 – 8.5	5.5 – 7.5	6.0 – 8.9
Optimum	6.0 – 7.5	6.0 – 6.5	6.5 – 8.0
Yield	Good	Fair	Medium
Establishment	Easy	Moderate	Difficult
Persistence	Poor	Medium	Very good
Tolerance to:			
Water logging	Fair	Good	Poor
Drought	Medium-poor	Medium-poor	Good
Anthelmintic	No	Low	Yes
Anti-bloating	No	Yes	Yes
Oestrogenic	Yes	No	No
Bioactive constituents	Polyphenol oxidase	Tannins	Tannins; oxidase

#### *Plant secondary metabolites for increasing the efficiency of ruminal protein digestion*

The role of condensed tannins in reducing ruminal protein degradation has been well documented (Jones and Mangan, 1977; Waghorn, 2008). A meta-analysis by Min *et al.* (2003) showed that increasing the concentration of condensed tannins progressively increased the amount of undegraded feed protein flowing into the duodenum without affecting microbial flow. By forming complexes with dietary proteins, condensed tannins generally slow the rate of protein degradation during fermentation in the rumen and during ensiling (Mueller-Harvey, 2006). Moreover, most plant proteases are located in the vacuole - just as condensed tannins are. Therefore, it is likely that, during the initial stages of digestion, condensed tannins may also reduce autolysis simply by complexing these enzymes (Kingston-Smith *et al.*, 2010). However, what is not yet fully understood is which types of condensed tannins (or plant features) create optimal degradation rates. For example, high concentrations of condensed tannins in trefoils (*Lotus pedunculatus* Cav., var. Maku) may be too 'potent' as ruminants cannot utilize its dietary protein fully as evidenced by high faecal-N contents (Waghorn *et al.*, 1998). However, in a few cases (birdsfoot trefoil, var. Goldi, and some sainfoin accessions), dietary protein appears to be appropriately protected by condensed tannins from ruminal degradation and available for post-ruminal digestion (Waghorn, 2008). Previous research indicated that this protective effect could not be transferred from sainfoin plants containing condensed tannins to red clover, which is free of condensed tannins (Beever and Siddons, 1984). Subsequent research found, however, that red clover contains polyphenol oxidase (PPO), which can generate covalent bonds between protein and polyphenols when

cells disintegrate, and this probably precluded any additional benefits from the condensed tannins in sainfoin. Promising results have been obtained by co-ensiling sainfoin and lucerne. This improved not only fermentation in laboratory silos, but more importantly it increased digestibility in sheep (Wang *et al.*, 2007). Synergistic effects have also been observed during *in vitro* fermentation of sainfoin and cocksfoot (*Dactylis glomerata* L.) (Niderkorn *et al.*, 2013).

The increased amount of duodenal N flow, associated with the presence of condensed tannins, is rarely matched by a greater utilization of amino acids in the intestine (Egan and Ulyatt, 1980; Aufrère *et al.*, 2008). When ruminants eat tanniniferous legumes, they excrete less urinary nitrogen and slightly more faecal nitrogen compared to other iso-nitrogenous diets. This is important since urinary urea is quickly converted to ammonia and nitrous oxide (N<sub>2</sub>O) and faecal nitrogen is more likely to contribute to soil organic matter (Mueller-Harvey, 2006; Woodward *et al.*, 2009).

Few studies have investigated the effects of legumes containing condensed tannins on milk yield under European conditions; however, a study from New Zealand found higher milk yields in dairy cows when feeding increasing proportions of birdsfoot trefoil in perennial ryegrass diets (Woodward *et al.*, 2009). In contrast to the USA, Canada and New Zealand, hardly any plant breeding programmes in Europe have involved legumes containing condensed tannins and it is, therefore, not surprising that Western Europe has only a few isolated areas where they are still grown.

Relatively little attention has also been paid to plant proteases, which appear to be active during the early stages of ruminal digestion (Kingston-Smith *et al.*, 2010). Concentrations of plant proteases differ two-fold among legumes and, together with other features, contributed to twenty-fold differences in protein half-lives in a simulated, but microorganism-free, rumen environment (a half-life of 19 h in sainfoin *versus* 1 h in white clover) (Kingston-Smith *et al.*, 2003). A few legumes also contain other features that are worth exploring. Red clover contains polyphenol oxidase, which can lead to covalently linked polyphenols and proteins. The resulting complex protects protein from rapid ruminal degradation, which may generate nutritional benefits for ruminants (Kingston-Smith *et al.*, 2010). Polyphenol oxidase potentially reduces ruminal proteolysis (Jones *et al.*, 1995) but *in vivo* experimental evidence for the positive effect of polyphenol oxidase on ruminal digestion is still lacking.

#### *Plant secondary metabolites for improving animal health while reducing medication*

Polyphenols and condensed tannins offer several opportunities to farmers for managing the health of their herds and flocks (Wang *et al.*, 2012). For instance, bloat is a serious digestive disorder which causes painful suffering or death to animals plus financial losses to farmers. It generally occurs when readily digestible plants degrade too fast in the rumen; this produces a stable proteinaceous foam that traps fermentation gases, which can no longer be eructed by animals (Wang *et al.*, 2012). However, plants containing condensed tannins, such as sainfoin, birdsfoot trefoil, crownvetch (*Coronilla varia* L.) and cicer milkvetch (*Astragalus cicer* L.), on their own or in mixtures with potentially bloat-forming forages never cause bloat (Mueller-Harvey, 2006). McMahon *et al.* (2000) showed that using fresh sainfoin as a complement to grazed lucerne helps to prevent bloat in cattle.

*Coccidia* cause diarrhoea and can result in serious economic losses. Recent research has obtained promising results from *in vitro* and *in vivo* studies with sainfoin in sheep (Saratsis *et al.*, 2012). In addition, condensed tannins are effective against fly-strike in sheep, which occurs when sheep are affected by wet faeces. Fly-strike can be controlled with forages containing condensed tannins, as these yield drier faeces, which in turn prevent flies from depositing their eggs on sheep (Waghorn, 2008).

An area that is currently receiving much attention concerns the use of secondary plant metabolites against parasitic worms, which are now a worldwide threat to animal welfare and production. Nematode resistance against all three classes of broad-spectrum anthelmintic drugs is challenging conventional treatments (Molento, 2009). Some farmers in the USA already rely on the legume, *Lespedeza cuneata* G. Don, rather than on veterinary drugs to control the *Haemonchus contortus* worm (Burke *et al.*, 2012). Condensed tannins represent a relatively untapped natural resource and can modulate nematode biology at key life-cycle stages (Hoste *et al.*, 2006). It is of particular interest that the anthelmintic bioactivity was still present or even enhanced after sainfoin was conserved as hay and silage (Hoste *et al.*, 2006; Häring *et al.*, 2008). Thus, sainfoin can be fed when it is needed most, before and after parturition when host immunity of the mother and new-born is low. Numerous studies have shown that flavanol monomers and condensed tannins are effective *in vitro* against parasitic nematodes from sheep, goats, cattle, deer and other species (Molan *et al.*, 2003; Novobilský *et al.*, 2011). Positive results have also been obtained *in vivo* (Min *et al.*, 2003; Häring *et al.*, 2008; Burke *et al.*, 2012; Azuhwi *et al.*, 2013). Condensed tannins are thought to act directly against the parasites because of their ability to form strong complexes with proline-rich proteins, which are present on nematode surfaces (Mueller-Harvey, 2006). Recent studies have also shown the potential for indirect effects because condensed tannins can stimulate the immune response in T-cells (Provenza and Villalba, 2010; Tibe *et al.*, 2012). This is particularly important as helminths are inherently immune-suppressive and down-regulate or inappropriately skew the host immune response (Maizels and Yazdanbakhsh, 2003).

### **Forage legume-based systems have potential for reducing the negative effects of livestock systems on the environment**

The great opportunities of legumes for environmentally friendly yet productive grassland-livestock systems derive from the different features reviewed above: (i) increased yield, (ii) replacement of industrial-N fertilizer by symbiotically-fixed N<sub>2</sub>, (iii) higher nutritive value and voluntary intake of forage and (iv) greater livestock performance. Taken together, all of these effects create important environmental advantages of legume-based grassland-husbandry systems. These advantages are evident not only at the sward level, but also at the whole-farm level. In addition, the benefits apply also to the functional unit of managed land area and to the functional unit of the final product.

#### *Forage legumes can reduce nitrate leaching*

At the sward level, the sometimes very high N inputs to the ecosystem from symbiosis can result in a risk of nitrate leaching (Hooper and Vitousek, 1997; 1998; Scherer-Lorenzen *et al.*, 2003; Palmberg *et al.*, 2005). Loiseau *et al.* (2001) reported higher annual leaching losses of N from lysimeters when swards were sown with pure white clover (28 to 140 kg N ha<sup>-1</sup>) than with pure perennial ryegrass (1 to 10 kg N ha<sup>-1</sup>). Much higher values are reported for bare soils (84 to 149 kg N ha<sup>-1</sup>). However, as long as the percentage of grass in mixed grass-legume swards is sufficient to take up mineral-N from the soil, it can be expected that this will prevent N losses by leaching. Under a mowing regime, Nyfeler (2009) found an increased risk of leaching of nitrate only if the percentage of legumes in the mixture was above 60-80% and this was combined with 150 kg N fertilizer input ha<sup>-1</sup> yr<sup>-1</sup>. Nevertheless, few studies have assessed the changes in nitrate content in the soil under mixtures containing legumes during periods of more than a few years. In the longer term, soil nitrate content could rise due to the mineralization of nitrogen-rich legume residues. In a five-year experiment, Oelmann *et al.* (2011) observed a positive effect of the presence of legumes on the NO<sub>3</sub>-N content in the soil,

but this effect did not increase with time, and therefore did not indicate an increasing risk of N leaching over the 5 years.

It has been suggested that a higher proportion of white clover in perennial ryegrass pastures, at the expense of mineral-N fertilizer, is an important component of low-input sustainable systems for livestock production (Thomas, 1992; Pflimlin *et al.*, 2003). Evidence comes not only from cut plots (above) but leaching of nitrate was also lower under grazing of mixed white clover-grass swards compared with highly fertilized pure grass swards (Hooda *et al.*, 1998; Ledgard *et al.*, 2009; Peyraud *et al.*, 2009). These results are explained mainly because mixed pastures do not support as high stocking rates as fertilized grass pastures and, to a lesser extent, by the down-regulation of symbiotic N<sub>2</sub> fixation under high mineral-N availability. At a similar stocking rate (3.3 cows ha<sup>-1</sup>) and milk yield per hectare (13,200 kg milk ha<sup>-1</sup>), Ledgard *et al.* (2009) reported similar leaching of N (30 kg N ha<sup>-1</sup>) under mixed grass-clover pastures and pure grass fertilized with 160 kg N ha<sup>-1</sup>, whereas N leaching increased to 60 kg N ha<sup>-1</sup> for a more intensively fertilized grass pasture (207 kg N fertilizer ha<sup>-1</sup>, 15,500 kg milk ha<sup>-1</sup>). Also, Vertès *et al.* (1997) found a 5 to 10% reduction of NO<sub>3</sub> leaching under grass-clover, compared to fertilized pure-grass pastures. Losses of nitrate under grazed grass-clover swards can rise with increasing proportions of clover (Schils, 1994; Ledgard *et al.*, 1999). There is less information available for other legumes. Losses through leaching were lower under pastures with a lucerne-grass mixture than from a white clover-grass mixture (Russelle *et al.*, 2001) for a similar yield.

At the level of the whole-farm system, despite an apparently negative effect on N excretion by ruminants, legumes actually provide opportunities for reducing N losses. For example, N-use efficiency decreases with the application of increasing amounts of mineral-N fertilizer (Scholefield *et al.*, 1991) and legumes overcome the need for a precise and timely supply of mineral-N fertilizer and hence reduce the amount of available ammonia N in the soil (Jarvis and Barraclough, 1991).

#### *Forage legumes can contribute to reduced greenhouse gas emissions*

##### Methane

Methane produced in the rumen is a large contributor to the greenhouse gas (GHG) emissions by livestock systems (Tamminga *et al.*, 2007; Waghorn and Hegarty, 2011). Legumes can contribute to reducing ruminal methane production per unit of intake. Ruminants fed legume forages generally emitted less methane than grass-fed animals, per unit of feed intake (McCaughy *et al.*, 1999; Waghorn *et al.*, 2006) although not in all cases (Van Dorland *et al.*, 2007). This may be due to a modification of the ruminal fermentation pattern toward propionate, which in turn is a hydrogen carrier and thus reduces the amount of methane produced. Inconsistency of results between experiments can arise from difference in forage composition (stage of maturity, presence of condensed tannins) and animal genotypes.

Condensed tannins may also be useful for reducing greenhouse gases (Kingston-Smith *et al.*, 2010) as several studies have shown that condensed tannins reduced methane production *in vitro*. A recent meta-analysis revealed a general anti-methanogenic effect of condensed tannins above 20 g kg<sup>-1</sup> DM in feeds (Jayanegara *et al.*, 2012). Some effects of condensed tannins were also reported from *in vivo* studies with sainfoin (Waghorn, 2008), birdsfoot trefoil (Woodward *et al.*, 2004) and sulla (Woodward *et al.*, 2002). It would appear that the anti-methanogenic properties of condensed tannins stem either from direct effects against methanogens and/or from indirect effects on protozoa. Interestingly, there were marked differences in the selectivities of different tannins (Pellikaan *et al.*, 2011). Preliminary results suggest that polymer size of condensed tannins is an important structural feature for anti-methanogenic activity (Tavendale *et al.*, 2005).

### Nitrous oxide

Each kg of N as ammonium nitrate produced in the industrial Haber-Bosch process consumes large amounts of energy (58 MJ) and also emits significant amounts of greenhouse gases (8.6 kg CO<sub>2</sub> equivalents) thereof 19 g N<sub>2</sub>O (ecoinvent, 2010). In addition, the IPCC (2006) suggested that for every 100 kg of N fertilizer added to the soil, on average 1.0 kg of N is emitted as N<sub>2</sub>O, which is a greenhouse gas that is *ca.* 300 times more active than CO<sub>2</sub> (Kingston-Smith *et al.*, 2010). The process of denitrification is the most important source of N<sub>2</sub>O from pasture systems (Soussana *et al.*, 2010). Denitrification occurs when the soil is wet, oxygen availability is restricted and nitrate concentration is high. Nitrification is favoured by a supply of ammonium-N in well-drained soils. Thus, large peaks of N<sub>2</sub>O emissions are measured in grasslands immediately after N-fertilizer applications (Ineson *et al.*, 1998; Klumpp *et al.*, 2011). There are three reasons why N<sub>2</sub>O emissions from legume-based grassland systems should be lower than from fertilized grass systems: (i) nitrogen is fixed symbiotically within the legume nodules and thus is not freely available in the soil in a reactive form, (ii) symbiotic N<sub>2</sub> fixation activity is down-regulated if the sink of N for plant growth is small and (iii) in optimized grass-legume mixtures the grass roots take up N derived from legume roots and from mineralization of soil organic matter. Indeed, a compilation by Jensen *et al.* (2012) showed that annual N<sub>2</sub>O emissions were largest in N fertilized grass swards (19 site-years; 4.49 kg N<sub>2</sub>O-N ha<sup>-1</sup>) followed by pure legume stands (17 site-years; 0.79 and 1.99 kg N<sub>2</sub>O-N ha<sup>-1</sup> for white clover and lucerne, respectively) and mixed grass-clover swards (8 site-years; 0.54 kg N<sub>2</sub>O-N ha<sup>-1</sup>). Within the revised greenhouse gas guidelines (IPCC, 2006), symbiotic N<sub>2</sub> fixation has actually been removed as a direct source of N<sub>2</sub>O because of a lack of evidence of significant emissions arising from the fixation process itself (Rochette and Janzen, 2005). These authors concluded that the N<sub>2</sub>O emissions induced by the growth of legume crops/forages may be estimated solely as a function of the above-ground and below-ground nitrogen inputs from crop/forage residue during pasture renewal. Emissions of N<sub>2</sub>O from legumes do occur as a result of the decomposition of residues from leguminous plants but the magnitude of such emissions remains uncertain (Baggs *et al.*, 2000).

At the level of the whole livestock system, Ledgard *et al.* (2009) and Basset-Mens *et al.* (2009) showed by using life-cycle analysis, that greenhouse gas emissions decreased by 1.15 to 1.00 kg eq-CO<sub>2</sub> kg<sup>-1</sup> milk with mixed grass-clover pastures compared to pure grass pastures because of the reduction of N<sub>2</sub>O emissions in New Zealand dairy farms. Basset-Mens *et al.* (2005) have compared greenhouse gas emissions from dairy farm systems in Sweden, southern Germany and New Zealand using life-cycle analysis and emission coefficients. The New Zealand system relies essentially on permanent grass-white clover pastures, which are grazed all year round with an annual N fertilizer input of 100 kg ha<sup>-1</sup> and less than 10% of the feed requirement of cows is provided by feed supplements. They showed that the total emission per 1 kg milk is 30 to 80% lower from the New Zealand system. Greenhouse gas emissions are high from intensive European dairy farms based on predominant grass pastures; the contribution of methane is reduced in proportion and CO<sub>2</sub> emissions were much higher in proportion (i.e. 3.7 times higher than from the New Zealand system), because of the production and transport of feed concentrates and mineral-N fertilizer and because of effluent management. Schils *et al.* (2005) compared the total emissions from dairy systems in the Netherlands, which were either fertilized ryegrass or grass-clover pastures (i.e. inputs of 208 and 17 kg mineral-N ha<sup>-1</sup> yr<sup>-1</sup>): greenhouse gas emissions kg<sup>-1</sup> of milk were 20% lower for grass-clover pasture-based systems.

## Carbon dioxide

Industrial production of each kg of inorganic N emits 2.25 kg of CO<sub>2</sub>. Legumes offer a big advantage because the entire C needed for symbiotic N<sub>2</sub> fixation comes directly from the atmosphere via photosynthesis and, thus, are 'greenhouse-gas neutral'.

A further option to mitigate climate change is C sequestration into the soil. New C can only be introduced into the soil via photosynthesis by plants and the C:N ratio of soil organic matter is fairly constant in almost all soils (Kirkby *et al.*, 2011). Consequently, C sequestration into soil organic matter ultimately means sequestration of N into soil organic matter (80 kg N t<sup>-1</sup> of C). Current evidence suggests that humus formation is particularly limited by the availability of N (Christopher and Lal, 2007). This again points to the importance of legumes and their symbiotic N<sub>2</sub> fixation for coupling C and N cycles and for delivering the N needed to sequester C into soil organic matter. Data from a large survey of soil organic matter in France (Arrouays *et al.*, 2001), and models (Soussana *et al.*, 2004), show that the conversion of short-term N-fertilized grass leys into grass-legume mixtures could sequester C into soil organic matter. Indeed, several studies found higher soil organic matter contents under grass-legume mixed swards than under pure grass swards (Ruz-Jerez *et al.*, 1994; Mortensen *et al.*, 2004).

### *Forage legumes reduce consumption of non-renewable energy*

The introduction of legumes reduces non-renewable energy consumption in livestock farms since they use atmospheric N and since no direct financial or energetic cost is linked to this N input. In comparison, each kg of inorganic N produced in the industrial Haber-Bosch process consumes large amounts of energy. The estimations are highly variable and range from 44 MJ (Kaltschmitt and Reinhardt, 1997) to 78 MJ (Kitani, 1999). Studies estimated that under French conditions 0.17 MJ of energy are required to produce 1 MJ of net energy with ryegrass fertilized at 150 kg N ha<sup>-1</sup>, but only 0.06 MJ with a ryegrass-white clover mixture, and 0.13 MJ for maize silage planted after wheat (Besnard *et al.*, 2006). Similarly, energy consumption decreased from 5.0 MJ kg<sup>-1</sup> milk for intensive dairy farms in the Netherlands to 4.0 MJ kg<sup>-1</sup> milk for French farms using maize silage and fertilized grasses and to 3.1 and 1.4 MJ kg<sup>-1</sup> for systems based on grazing in Ireland and New Zealand, respectively (Le Gall *et al.*, 2009; Peyraud *et al.*, 2009). The higher energy consumption in Irish grassland-based systems appears to be linked to the utilization of high amounts of N fertilizer on pure ryegrass pastures, in comparison with the lower use of N in New Zealand systems.

### **Legumes offer an option for adapting to atmospheric change**

Legumes – again through their coupling of the C and N cycles – provide a useful option for adapting to atmospheric change. Elevated atmospheric CO<sub>2</sub> concentrations stimulate photosynthesis and this leads to a one-sided increase in C availability within the ecosystem. Research has shown that, under controlled conditions and ample nutrient supply, the yield response of plants follows the increased rate of photosynthesis. However, under field conditions, N is the major limiting factor in the yield response of grasslands to elevated CO<sub>2</sub>. Thus, elevated atmospheric CO<sub>2</sub> concentrations resulted in a decrease in the index of N nutrition of grasses (Soussana and Hartwig, 1996; Zanetti *et al.*, 1997), which indicates an increased N limitation of growth. Legumes, with their access to the unlimited N source of the atmosphere, have the potential to close such an increased gap between N demand and N availability of the ecosystem. Indeed, in fertile grasslands, legumes benefit more from elevated atmospheric CO<sub>2</sub> concentrations than non-fixing species (Hebeisen *et al.*, 1997; Lüscher *et al.*, 1998; 2000; Campbell *et al.*, 2000) and this results in a significant increase in symbiotic N<sub>2</sub> fixation due to higher proportions of legumes in the sward and due to a higher



proportion of N derived from symbiosis in the legume plant (Soussana and Hartwig, 1996; Zanetti *et al.*, 1997). In fact, the additional N harvested under high atmospheric CO<sub>2</sub> concentrations was derived solely from increased activity of symbiotic N<sub>2</sub> fixation (reviewed in Lüscher *et al.*, 2004; Soussana and Lüscher, 2007).

There are other reasons why legumes can be suggested to be well adapted to future climatic conditions. Legumes have higher temperature requirements for growth than their companion grasses (Mitchell, 1956; Davies and Young, 1967). Warmer temperatures should, thus, result in a competitive advantage for the legumes as indicated by the seasonal cycle of the white clover proportion in mixed swards, which is high in the summer and low in spring and autumn (Lüscher *et al.*, 2005). Especially in temperature-limited environments of high altitudes and high latitudes, the projected increase in temperature could result in an advantage for legumes. The projected increase in the frequency and severity of drought-stress periods may increase interest in the use of deep-rooting species such as red clover, lucerne, birdsfoot trefoil and sainfoin, as they allow the use of water reserves in deeper soil layers. In addition, niche theories not only predict higher yields of mixed swards compared to monocultures, but also that they can better deal with climatic variability and stress and that they show higher resilience after cessation of stress (insurance hypothesis; Naeem and Li, 1997; Yachi and Loreau, 1999). Accordingly, drought-stress vulnerability and resilience of deep (red clover, sainfoin) and shallow rooting (white clover) legumes and of grass-legume mixtures are investigated in the projects AnimalChange ([www.animalchange.eu](http://www.animalchange.eu)) and LegumePlus ([www.legumeplus.eu](http://www.legumeplus.eu)).

### **Technology needs for keeping a more stable abundance of legume in the sward**

In conclusion, grass-clover mixtures with 30 to 50% of legumes seem to be an optimal system: they yield high amounts of N from symbiosis, generate high forage yields of high nutritive value, which generates high voluntary intakes and livestock performances and, at the same time, they minimize the risk of N losses to the environment. The big challenge for legume-based grassland-husbandry systems, however, will be to maintain the proportion of legumes within this optimal range.

Legumes have a distinct competitive advantage in N-limited systems (Hartwig, 1998). When competing with non-fixers, legumes avoid N deprivation by supplementing mineral-N uptake with symbiotic N<sub>2</sub> fixation, thereby retaining a relatively high growth rate even in a low soil-N environment (Woledge, 1988). In contrast, where mineral-N is abundant, N<sub>2</sub> fixation is energetically costly and N<sub>2</sub> fixers tend to be competitively excluded by non-fixing species (Faurie *et al.*, 1996; Soussana and Tallec, 2010). There was a strong decrease in the proportion of legumes in the swards of the pan-European experiment in its third and final year (Nyfeler *et al.*, 2009; Finn *et al.*, 2013) and this further confirms the difficulties of maintaining the desired abundance of legumes in mixtures (Frame, 1986; Guckert and Hay, 2001). Sward management strategies with reduced N fertilizer input and/or increased cutting frequencies can increase the proportion of white clover (Schwank *et al.*, 1986). The effectiveness of such management treatments to regulate the proportion of white clover is evident from the Swiss Free-Air CO<sub>2</sub> Enrichment (FACE) experiment (Hebeisen *et al.*, 1997; Zanetti *et al.*, 1997). Averaged over the first three years, the contribution of white clover was 14% at infrequent defoliation combined with high N fertilization, whereas it was 57% at frequent defoliation combined with low levels of N fertilizer. However, due to large seasonal variations, there were also periods with unsustainably high clover percentages of above 80% (Lüscher *et al.*, 2005).

Another option to optimize and stabilize legume abundance in mixtures is an optimized composition of seed mixtures. This will require decisions on how many and which species to include, and which proportions of the species and which cultivar of the species to choose.

This option is evident from the Swiss site of the pan-European experiment, where, besides the experimental four-species mixtures (Kirwan *et al.*, 2007; Nyfeler *et al.*, 2009), Swiss Standard Mixtures (Suter *et al.*, 2012) were also examined. These mixtures contain more species (up to eight) and their composition (relative and absolute abundance of species) was continuously ameliorated during the last few decades based on experiments and observations on farms. Over the three years of the experiment, the decline in clover abundance in the Swiss Standard Mixtures was much smaller than that in the four-species mixtures of the pan-European experiment (Suter *et al.*, 2010). Development of seed mixtures containing species with comparable competitive abilities could result in more balanced and stable mixtures (Lüscher *et al.*, 1992). Moreover, not only do species differ in their competitive abilities but also cultivars within species. Suter *et al.* (2007) found that the realized species composition of the established sward differed tremendously depending on which cultivars were chosen for the seed mixture. All these results demonstrate that the composition of the seed mixture offers a multi-factorial opportunity for optimization.

## Conclusions

As a component of mixed grass-legume swards, forage legumes offer important opportunities for tackling future agricultural challenges. The great potential of legumes for sustainable intensification is related not just to one specific feature; their strength stems from the fact that several of their features can act together on different 'sites' in the soil-plant-animal-atmosphere system. It is interesting to note that their advantages are most pronounced in mixed swards with 30-50% of legumes. These advantages are: (i) increased forage production; (ii) 'greenhouse gas-neutral' and 'energy-neutral' N input into grasslands via symbiotic N<sub>2</sub> fixation; (iii) support of non-N<sub>2</sub>-fixing plants in the grassland through transfer of symbiotically fixed N; (iv) higher nutritive value and voluntary intake of the forage with a less marked decline of quality with advancing maturity than grasses, which lead to (v) higher livestock performance; in addition, bioactive plant secondary metabolites of legumes can enhance (vi) efficiency of protein digestion by ruminants and (vii) benefit animal health through lower medication. These multiple advantages benefit the whole grassland-husbandry system through reduced dependency on fossil energy and industrial N fertilizer, lower nitrate and greenhouse gas emissions into the environment, lower production costs, higher productivity and protein self-sufficiency. In addition, legumes may offer an option for adapting to higher atmospheric CO<sub>2</sub> concentrations and to climate change. Legumes generate these benefits at the level of the land management unit and also at the level of the final product unit. However, legumes suffer from some limitations and future research is needed to fully exploit the opportunities they offer. The most important areas for research are: (i) more predictable and controllable proportions of legumes within mixed plant communities, which, most probably, is achievable through innovative management strategies, optimized seed mixtures and breeding for increased competitive ability and/or niche complementarity; (ii) improved nutritive value of fresh forage and, especially, silage, which can be addressed by optimizing the energy/protein balance within the plants (e.g. by increasing water-soluble carbohydrate concentration); (iii) better exploitation of the multiple opportunities offered by plant secondary metabolites, which requires knowledge of optimum structures and concentrations of these compounds, and development of cultivars and cultivation techniques that enable farmers to produce these optimized plant secondary metabolites reliably. The development of legume-based systems of grassland husbandry undoubtedly constitutes one of the pillars for more sustainable and competitive ruminant production systems and it can only be expected that legumes will become more important in the future.

## Acknowledgements

The publication was supported by the European Community's Seventh Framework Programme (FP7/2007-2013) under the grant agreements FP7-266018 (AnimalChange), FP7-244983 (MultiSward), PITN-GA-2011-289377 (LegumePlus) and FP7-245216 (Legume-Futures). We are grateful to J. Nösberger, M. Suter and O. Huguenin-Elie for helpful discussions.

## References

- Abberton M.T. and Marshall A.H. (2010) White clover. In: Boller B., Posselt U.K. and Veronesi F. (eds) *Fodder Crops and Amenity Grasses, Handbook of Plant breeding*, Vol. 5. Springer, New York, USA, pp. 457-476.
- Aigner I. (2009) Our future grows in the country. Stuttgart: Federal Agriculture Minister. Available at: <http://www.bmelv.de/SharedDocs/Reden/EN/2009/07-02-German-Farmers-Day.html> (accessed on 3 January 2013).
- Alder F.E. and Minson D.J. (1963) The herbage intake of cattle grazing lucerne and cocksfoot pasture. *The Journal of Agricultural Science* 60, 359-369.
- Arnaud J.D., Le Gall A. and Pflimlin A. (1993) Evolution des surfaces en légumineuses fourragères en France. *Fourrages* 134, 145-154.
- Arrouays D., Deslais W. and Bateau V. (2001) The carbon content of topsoil and its geographical distribution in France. *Soil Use and Management* 17, 7-11.
- Aufrère J., Dudilieu M. and Poncet C. (2008) *In vivo* and *in situ* measurements of the digestive characteristics of sainfoin in comparison with lucerne fed to sheep as fresh forages at two growth stages and as hay. *Animal* 2, 1331-1339.
- Azuhnwi B.N., Boller B., Martens M., Dohme-Meier F., Ampuero S., Günter S., Kreuzer M. and Hess H.D. (2011) Morphology, tannin concentration and forage value of fifteen Swiss accessions of sainfoin (*Onobrychis viciifolia* Scop.) as influenced by harvest time and cultivation site. *Grass and Forage Science* 66, 474-487.
- Azuhnwi B.N., Hertzberg H., Arrigo Y., Gutzwiller A., Hess H.D., Mueller-Harvey I., Torgerson P., Kreuzer M. and Dohme-Meier F. (2013) Investigation of sainfoin (*Onobrychis viciifolia*) cultivar differences on nitrogen balance and fecal egg count in artificially infected lambs. *Journal of Animal Science* doi:10.2527/jas.2012-5351.
- Baggs E.M., Rees R.M., Smith K.A. and Vinten A.J.A. (2000) Nitrous oxide emission from soils after incorporating crop residues. *Soil Use and Management* 16, 82-87.
- Basset-Mens C., Ledgard S. and Carran A. (2005) First life cycle assessment of milk production from New Zealand dairy farm systems. [www.anzsee.org/anzsee2005papers/Basset-Mens\\_LCA\\_NZ\\_milk\\_production.pdf](http://www.anzsee.org/anzsee2005papers/Basset-Mens_LCA_NZ_milk_production.pdf) (accessed on 3 January 2013).
- Basset-Mens C., Ledgard S. and Boyes M. (2009) Eco-efficiency of intensification scenarios for milk production in New Zealand. *Ecological Economics* 68, 1615-1625.
- Beever D.E. and Siddons R.C. (1984) Digestion and metabolism in the grazing ruminant. In: Milligan L.P., Grovum W.L. and Dobson A. (eds) *Control of Digestion and Metabolism in Ruminants*. Proceedings of the 6<sup>th</sup> International Symposium on Ruminant Physiology, Banff (Canada), Prentice-Hall, Englewood Cliffs, N.J. (USA), pp. 479-497.
- Beever D.E., Dhanoa M.S., Losada H.R., Evans R.T., Cammell S.B. and France J. (1986) The effect of forage species and stage of harvest on the process of digestion occurring in the rumen of cattle. *British Journal of Nutrition* 56, 439-454.
- Besnard A., Montarges-Lellahi A. and Hardy A. (2006) Système de culture et nutrition azotée. Effets sur les émissions de GES et le bilan énergétique. *Fourrages* 187, 311-320.
- Boller B., Schubiger F.X. and Kölliker R. (2010) Red clover. In: Boller B., Posselt U.K. and Veronesi F. (eds) *Fodder Crops and Amenity Grasses, Handbook of plant breeding*, Vol 5. Springer, New York, USA, pp. 439-456.
- Boller B.C. and Nösberger J. (1987) Symbiotically fixed nitrogen from field-grown white and redclover mixed with ryegrasses at low levels of <sup>15</sup>N-fertilization. *Plant and Soil* 104, 219-226.
- Bowman W. D., Schardt J. C. and Schmidt S. K. (1996) Symbiotic N<sub>2</sub>-fixation in alpine tundra: ecosystem input and variation in fixation rates among communities. *Oecologia*, 108, 345-350.
- Brito A.F., Tremblay G.F., Bertrand A., Castonguay Y., Bélanger G., Michaud R., Lapierre H., Benchaar C., Petit H.V., Ouellet D.R. and Berthiaume R. (2008) Alfalfa cut at sundown and harvested as baleage improves milk yield of late-lactation dairy cows. *Journal of Dairy Science* 91, 3968-3982.
- Brown L., Scholefield D., Jewkes E.C., Preedy N., Wadge K. and Butler M. (2000) The effect of sulphur application on the efficiency of nitrogen use in two contrasting grassland soils. *The Journal of Agricultural Science* 135 131-138.

- Burke J.M., Miller J.E., Mosjidis J.A. and Terrill T.H. (2012) Grazing sericea lespedeza for control of gastrointestinal nematodes in lambs. *Veterinary Parasitology* 186, 507-512.
- Campbell B.C., Stafford Smith D.M., Ash A.J., Fuhrer J., Gifford R.M., Hiernaux P., Howden S.M., Jones M.B., Ludwig J.A., Manderscheid R., Morgan J.A., Newton P.C.D., Nösberger J., Owensby C.E., Soussana J.F., Tuba Z. and ZuoZhong C. (2000) A synthesis of recent global change research on pasture and rangeland production: reduced uncertainties and their management implications. *Agriculture, Ecosystems and Environment* 82, 39-55.
- Cardinale B.J., Wright J.P., Cadotte M.W., Carroll I.T., Hector A., Srivastava D.S., Loreau M. and Weis J.J. (2007) Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proceedings of the National Academy of Sciences of the United States of America* 104, 18123-18128.
- Carlsson G. and Huss-Danell K. (2003) Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil* 253, 353-372.
- Carlsson G., Palmborg C., Jumpponen A., Scherer-Lorenzen M., Högberg P. and Huss-Danell K. (2009) N<sub>2</sub> fixation in three perennial *Trifolium* species in experimental grasslands of varied plant species richness and composition. *Plant Ecology* 205, 87-104.
- Castle M.E., Reid D. and Watson J.N. (1983) Silage and milk production: studies with diets containing white clover silage. *Grass and Forage Science* 38, 193-200.
- Chenais F. (1993) Ensilage de légumineuses et production laitière. *Fourrages* 134, 258-265.
- Christopher S.F. and Lal R. (2007) Nitrogen management affects carbon sequestration in North American cropland soils. *Critical Reviews in Plant Sciences* 26, 45-64.
- Cohen D.C., Stockdale C.R. and Doyle P.T. (2006) Feeding an energy supplement with white clover silage improves rumen fermentation, metabolisable protein utilisation and milk production in dairy cows. *Australian Journal of Agricultural Research* 57, 367-375.
- Connolly J., Bell T., Bolger T., Brophy C., Carnus T., Finn J.A., Kirwan L., Isbell F., Levine J., Lüscher A., Picasso V., Roscher C., Sebastia M.T., Suter M. and Weigelt A. (2013) An improved model to predict the effects of changing biodiversity levels on ecosystem function. *Journal of Ecology* 101, 344-355.
- Cralle H.T. and Heichel G.H. (1982) Temperature and chilling sensitivity of nodule nitrogenase activity in unhardened alfalfa. *Crop Science* 22, 300-304.
- Daepf M., Nösberger J. and Lüscher A. (2001) Nitrogen fertilization and developmental stage alter the response of *Lolium perenne* to elevated CO<sub>2</sub>. *New Phytologist* 150, 347-358.
- Davies W.E. and Young N.R. (1967) The characteristics of European, Mediterranean and other populations of white clover (*Trifolium repens* L.). *Euphytica* 16, 330-340.
- Delaby L. and Peccatte J.R. (2003) Valeur alimentaire des prairies d'association ray grass anglais/trèfle blanc utilisées entre 6 et 12 semaines de repousse. *Rencontres Recherches Ruminants* 10, 389.
- Dewhurst R.J., Fisher W.J., Tweed J.K.S. and Wilkins R.J. (2003) Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. *Journal of Dairy Science* 86, 2598-2611.
- Dewhurst R.J., Delaby L., Moloney A., Boland T. and Lewis E. (2009) Nutritive value of forage legumes used for grazing and silage. *Irish Journal of Agricultural and Food Research* 48, 168-187.
- Dixon R.A., Liu C. and Jun J.H. (2012) Metabolic engineering of anthocyanins and condensed tannins in plants. *Current Opinion in Biotechnology* 24, 329-335.
- ecoinvent Centre (2010) ecoinvent Data - The Life Cycle Inventory Data V2.2. Swiss Centre for Life Cycle Inventories, Dübendorf, Available at <http://www.ecoinvent.org> (accessed on 12 February 2013).
- Egan A.R. and Ulyatt M.J. (1980) Quantitative digestion of fresh herbage by sheep. *The Journal of Agricultural Science* 94, 47-56.
- European Commission (2010) The CAP towards 2020: meeting the food, natural resources and territorial challenges of the future. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions, Brussels, 18 November, COM(2010) 672 final.
- European Parliament (2011) The EU protein deficit: what solution for a long-standing problem? European Parliament, Brussels.
- Faurie O., Soussana J.F. and Sinoquet H. (1996) Radiation interception, partitioning and use in grass-clover mixtures. *Annals of Botany* 77, 35-45.
- Finn J.A., Kirwan L., Connolly J., Sebastia M.T., Helgadóttir Á., Baadshaug O.H., Bélanger G., Black A., Brophy C., Collins R.P., Čop J., Dalmannsdóttir S., Delgado I., Elgersma A., Fothergill M., Frankow-Lindberg B.E., Ghesquire A., Golinska B., Golinski P., Griep P., Gustavsson A.M., Höglind M., Huguénin-Elie O., Jørgensen M., Kadziulienė Ž., Kurki P., Llurba R., Lunnan T., Porqueddu C., Suter M., Thumm U. and Lüscher A. (2013) Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: a 3-year continental-scale field experiment. *Journal of Applied Ecology* 50, 365-375.

- Frame J. (1986) The production and quality potential of four forage legumes sown alone and combined in various associations. *Crop Research* 25, 103-122.
- Frame J. (1991) Herbage production and quality of a range of secondary grass species at five rates of fertilizer nitrogen application. *Grass and Forage Science* 46, 139-151.
- Frame J. (2005) *Forage Legumes for Temperate Grasslands*, Food and Agriculture Organization of the United Nations, Rome, Science Publishers Inc., Plymouth, UK, 309 pp.
- Galloway J.N., Townsend A.R., Erismann J.W., Bekunda M., Cai Z.C., Freney J.R., Martinelli L.A., Seitzinger S.P. and Sutton M.A. (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320, 889-892.
- Gately T.F. (1981) Evaluation of the role of white clover (cv Blanca) for milk production. *Winter Meeting British Grassland Society*, 5.1-5.3.
- Godfray H.C.J., Beddington J.R., Crute I.R., Haddad L., Lawrence D., Muir J.F., Pretty J., Robinson S., Thomas S.M. and Toulmin C. (2010) Food security: the challenge of feeding 9 billion people. *Science* 327, 812-818.
- Gruber N. and Galloway J.N. (2008) An earth-system perspective of the global nitrogen cycle. *Nature* 451, 293-296.
- Guckert A. and Hay R.K.M. (2001) The overwintering, spring growth, and yield in mixed species swards of white clover in Europe. *Annals of Botany* 88, 667-668.
- Häring D.A., Suter D., Amrhein N. and Lüscher A. (2007) Biomass allocation is an important determinant of tannin concentration in growing plants. *Annals of Botany* 99, 111-120.
- Häring D.A., Scharenberg A., Heckendorn F., Dohme F., Lüscher A., Maurer V., Suter D. and Hertzberg H. (2008) Tanniferous forage plants: agronomic performance, palatability and efficacy against parasitic nematodes in sheep. *Renewable Agriculture and Food Systems* 23, 19-29.
- Harris S.L., Auldred M.J., Clark D.A. and Jansen E.B.L. (1998) Effect of white clover content in the diet on herbage intake, milk production and milk composition of New Zealand dairy cows housed indoors. *Journal of Dairy Research* 65, 389-400.
- Hartwig U.A. (1998) The regulation of symbiotic N<sub>2</sub> fixation: a conceptual model of N feedback from the ecosystem to the gene expression level. *Perspectives in Plant Ecology, Evolution and Systematics* 1, 92-120.
- Hebeisen T., Lüscher A., Zanetti S., Fischer B.U., Hartwig U.A., Frehner M., Hendrey G.R., Blum H. and Nösberger J. (1997) Growth response of *Trifolium repens* L. and *Lolium perenne* L. as monocultures and bi-species mixture to free air CO<sub>2</sub> enrichment and management. *Global Change Biology* 3, 149-160.
- Heichel G.H. and Henjum K.I. (1991) Dinitrogen fixation, nitrogen transfer, and productivity of forage legume-grass communities. *Crop Science* 31, 202-208.
- Henry G.H.R. and Svoboda J. (1986) Dinitrogen fixation (acetylene reduction) in high-Arctic sedge meadow communities. *Arctic and Alpine Research* 18, 181-187.
- Herridge D.F., Peoples M.B. and Boddey R.M. (2008) Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil* 311, 1-18.
- Hill J. (1990) The three C's – competition, coexistence and coevolution – and their impact on the breeding of forage crop mixtures. *Theoretical and Applied Genetics* 79, 168-176.
- Hille Ris Lambers J., Harpole W.S., Tilman D., Knops J. and Reich P.B. (2004) Mechanisms responsible for the positive diversity-productivity relationship in Minnesota grasslands. *Ecology Letters* 7, 661-668.
- Hooda P.S., Moynagh M., Svoboda I.F. and Anderson H.A. (1998) A comparative study of nitrate leaching from intensively managed monoculture grass and grass-clover pastures. *The Journal of Agricultural Science* 131, 267-275.
- Hooper D.U. and Dukes J.S. (2004) Overyielding among plant functional groups in a long-term experiment. *Ecology Letters* 7, 95-105.
- Hooper D.U. and Vitousek P.M. (1997) The effects of plant composition and diversity on ecosystem processes. *Science* 277, 1302-1305.
- Hooper D.U. and Vitousek P.M. (1998) Effects of plant composition and diversity on nutrient cycling. *Ecological Monographs* 68, 121-149.
- Hooper D.U., Chapin F.S., Ewel J.J., Hector A., Inchausti P., Lavorel S., Lawton J.H., Lodge D.M., Loreau M., Naem S., Schmid B., Setälä H., Symstad A.J., Vandermeer J. and Wardle D.A. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75, 3-35.
- Hooper D.U., Adair E.C., Cardinale B.J., Byrnes J.E.K., Hungate B.A., Matulich K.L., Gonzalez A., Duffy J.E., Gamfeldt L. and O'Connor M.I. (2012) A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature* 486, 105-108.
- Hoste H., Jackson F., Athanasiadou S., Thamsborg S.M. and Hoskin S.O. (2006) The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends in Parasitology* 22, 253-261.
- Humphreys J., Casey I.A. and Laidlaw A.S. (2009) Comparison of milk production from clover-based and fertilizer-N-based grassland on a clay-loam soil under moist temperate climatic conditions. *Irish Journal of Agricultural and Food Research* 48, 189-207.

- Ineson P., Coward P.A. and Hartwig U.A. (1998) Soil gas fluxes of N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> beneath *Lolium perenne* under elevated CO<sub>2</sub>: the Swiss free air carbon dioxide enrichment experiment. *Plant and Soil* 198, 89-95.
- Institut de l'Élevage (2004) Associations graminées-trèfle blanc, le pâturage gagnant. Collection Synthèses, Technipiel, Paris, France, 64 p.
- INRA (2007) Alimentation des bovins, ovins et caprins. Besoins des animaux. Valeur des aliments. Tables INRA 2007. Editions Quae, Paris, France, 307 pp.
- IPCC (2006) Guidelines for National Greenhouse Gas Inventories. *Intergovernmental Panel on Climate Change*, Tokyo, Japan.
- Jacot K.A., Lüscher A., Nösberger J. and Hartwig, U.A. (2000a) The relative contribution of symbiotic N<sub>2</sub> fixation and other nitrogen sources to grassland ecosystems along an altitudinal gradient in the Alps. *Plant and Soil* 225, 201-211.
- Jacot K.A., Lüscher A., Nösberger J. and Hartwig, U.A. (2000b) Symbiotic N<sub>2</sub> fixation of various legume species along an altitudinal gradient in the Swiss Alps. *Soil Biology and Biochemistry* 32, 1043-1052.
- Jamot J. and Grenet E. (1991) Microscopic investigation of changes in histology and digestibility in the rumen of a forage grass and forage legume during the first growth stage. *Reproduction, Nutrition, Développement* 31, 441-450.
- Jarvis S.C. and Barraclough D. (1991) Variations in mineral nitrogen under grazed grassland swards. *Plant and Soil* 138, 177-188.
- Jayanegara A., Leiber F. and Kreuzer M. (2012) Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from *in vivo* and *in vitro* experiments. *Journal of Animal Physiology and Animal Nutrition* 96, 356-375.
- Jensen E.S., Peoples M.B., Boddey R.M., Gresshoff P.M., Hauggaard-Nielsen H., Alves B.J.R. and Morrison M.J. (2012) Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. *Agronomy for Sustainable Development* 32, 329-364.
- Jones B.A., Muck R.E. and Hatfield R.D. (1995) Red clover extracts inhibit legume proteolysis. *Journal of the Science of Food and Agriculture* 67, 329-333.
- Jones W.T. and Mangan J.L. (1977) Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia*) with fraction 1 leaf protein and with submaxillary mycoprotein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* 28, 126-136.
- Kaltschmitt M. and Reinhardt G.A. (1997) *Nachwachsende Energieträger - Grundlagen, Verfahren, ökologische Bilanzierung*, Vieweg Verlag, Braunschweig/Wiesbaden, Germany.
- Kessler W., Boller B.C. and Nösberger J. (1990) Distinct influence of root and shoot temperature on nitrogen fixation by white clover. *Annals of Botany* 65, 341-346.
- Kingston-Smith A.H., Bollard A.L., Shaw R.K., Davies T.E. and Theodorou M.K. (2003) Correlations between protein content and protease activity in forage crops. *Aspects of Applied Biology* 70, 101-106.
- Kingston-Smith A.H., Edwards J.E., Huws S.A., Kim E.J. and Abberton M. (2010) Plant-based strategies towards minimising 'livestock's long shadow'. *Proceedings of the Nutrition Society* 69, 613-620.
- Kirkby C.A., Kirkegaard J.A., Richardson A.E., Wade L.J., Blanchard C. and Batten G. (2011) Stable soil organic matter: a comparison of CNPS ratios in Australian and other world soils. *Geoderma* 163, 197-208.
- Kirwan L., Lüscher A., Sebastià M.T., Finn J.A., Collins R.P., Porqueddu C., Helgadóttir Á., Baadshaug O.H., Brophy C., Coran C., Dalmannsdóttir S., Delgado I., Elgersma A., Fothergill M., Frankow-Lindberg B.E., Golinski P., Grieu P., Gustavsson A.M., Höglind M., Huguenin-Elie O., Iliadis C., Jørgensen M., Kadziulienė Ž., Karyotis T., Lunnan T., Malengier M., Maltoni S., Meyer V., Nyfeler D., Nykanen-Kurki P., Parente J., Smit H.J., Thumm U. and Connolly J. (2007) Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology* 95, 530-539.
- Kirwan L., Connolly J., Finn J.A., Brophy C., Lüscher A., Nyfeler D. and Sebastià M.T. (2009) Diversity-interaction modeling: estimating contributions of species identities and interactions to ecosystem function. *Ecology* 90, 2032-2038.
- Kitani O., Jungbluth T., Peath R.M., and Ramdani A. (eds., 1999) CIGR handbook of agricultural engineering. Volume V: *Energy & Biomass Engineering*. American Society of Agricultural Engineers, St Joseph, USA.
- Klump K., Bloor J.M.G., Ambus P. and Soussana J.F. (2011) Effects of clover density on N<sub>2</sub>O emissions and plant-soil N transfers in a fertilized upland pasture. *Plant and Soil* 343, 97-107.
- Le Gall A., Beguin E., Dollé J.B., Manneville V. and Pflimlin A. (2009) Nouveaux compromis techniques pour concilier les impératifs d'efficacité économique et environnementale en élevage herbivore. *Fourrages* 198, 131-152.
- Ledgard S.F. and Steele K.W. (1992) Biological nitrogen-fixation in mixed legume/grass pastures. *Plant and Soil* 141, 137-153.
- Ledgard S.F., Penno J.W. and Sprosen M.S. (1999) Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. *The Journal of Agricultural Science* 132, 215-225.

- Ledgard S., Schils R., Eriksen J. and Luo J. (2009) Environmental impacts of grazed clover/grass pastures. *Irish Journal of Agricultural and Food Research* 48, 209-226.
- Lee M.R.F., Harris L.J., Moorby J.M., Humphreys M.O., Theodorou M.K., MacRae J.C. and Scollan N.D. (2002) Rumen metabolism and nitrogen flow to the small intestine in steers offered *Lolium perenne* containing different levels of water-soluble carbohydrate. *Animal Science* 74, 587-596.
- Li L., Li S.M., Sun J.H., Zhou L.L., Bao X.G., Zhang H.G. and Zhang F.S. (2007) Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 11192-11196.
- Loiseau P., Carrère P., Lafarge M., Delpy R. and Dublanchet J. (2001) Effect of soil-N and urine-N on nitrate leaching under pure grass, pure clover and mixed grass/clover pastures. *European Journal of Agronomy* 14, 113-121.
- Loreau M. and Hector A. (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature* 412, 72-76.
- Loreau M., Naeem S., Inchausti P., Bengtsson J., Grime J.P., Hector A., Hooper D.U., Huston M.A., Raffaelli D., Schmid B., Tilman D. and Wardle D.A. (2001) Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science* 294, 804-808.
- Lüscher A. and Jacquard P. (1991) Coevolution between interspecific plant competitors? *Trends in Ecology and Evolution* 6, 355-358.
- Lüscher A., Connolly J. and Jacquard P. (1992) Neighbour specificity between *Lolium perenne* and *Trifolium repens* from a natural pasture. *Oecologia* 91, 404-409.
- Lüscher A., Hendrey G.R. and Nösberger J. (1998) Long-term responsiveness to free air CO<sub>2</sub> enrichment of functional types, species and genotypes of plants from fertile permanent grassland. *Oecologia*, 113 37-45.
- Lüscher A., Hartwig U.A., Suter D. and Nösberger J. (2000) Direct evidence that symbiotic N<sub>2</sub> fixation in fertile grassland is an important trait for a strong response of plants to elevated atmospheric CO<sub>2</sub>. *Global Change Biology* 6, 655-662.
- Lüscher A., Daapp M., Blum H., Hartwig U.A. and Nösberger J. (2004) Fertile temperate grassland under elevated atmospheric CO<sub>2</sub> – role of feed-back mechanisms and availability of growth resources. *European Journal of Agronomy* 21, 379-398.
- Lüscher A., Fuhrer J. and Newton P.C.D. (2005) Global atmospheric change and its effect on managed grassland systems. In: McGilloway D.A. (ed) *Grassland: a global resource*, Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 251-264.
- Lüscher A., Finn J.A., Connolly J., Sebastià M.T., Collins R., Fothergill M., Porqueddu C., Brophy C., Huguenin-Elie O., Kirwan L., Nyfeler D. and Helgadóttir Á. (2008) Benefits of sward diversity for agricultural grasslands. *Biodiversity* 9, 29-32.
- Lüscher A., Soussana, J.F. and Huguenin-Elie O. (2011) Role and impacts of legumes in grasslands for high productivity and N gain from symbiotic N<sub>2</sub> fixation. In: Lemaire G., Hodgson J. and Chabbi A. (eds) *Grassland Productivity and Ecosystem Services*, CAB International, Wallingford, UK, pp. 101-107.
- Maizels R.M. and Yazdanbakhsh M. (2003) Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology* 3, 733-744.
- Marquard E., Weigelt A., Temperton V.M., Roscher C., Schumacher J., Buchmann N., Fischer M., Weisser W.W. and Schmid B. (2009) Plant species richness and functional composition drive overyielding in a six-year grassland experiment. *Ecology* 90, 3290-3302.
- McCaughy W.P., Wittenberg K. and Corrigan D. (1999) Impact of pasture type on methane production by lactating beef cows. *Canadian Journal of Animal Science* 79, 221-226.
- McMahon L.R., McAllister T.A., Berg B.P., Majak W., Acharya S.N., Popp J.D., Coulman B.E., Wang Y. and Cheng K.J. (2000) A review of the effect of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. *Canadian Journal of Plant Science* 80, 469-485.
- Miller L.A., Moorby J.M., Davis D.R., Humphreys M.O., Scollan N.D., MacRae J.C. and Theodorou M.K. (2001) Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. *Grass and Forage Science* 56, 383-394.
- Min B.R., Barry T.N., Attwood G.T. and McNabb W.C. (2003) The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Animal Feed Science and Technology* 106, 3-19.
- Mitchell K.J. (1956) Growth of pasture species under controlled environment. I. Growth at various levels of constant temperature. *New Zealand Journal of Science and Technology* 38A, 203-216.
- Molan A.L., Meagher L.P., Spencer P.A. and Sivakumaran S. (2003) Effect of flavan-3-ols on *in vitro* egg hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis*. *International Journal for Parasitology* 33, 1691-1698.
- Molento M.B. (2009) Parasite control in the age of drug resistance and changing agricultural practices. *Veterinary Parasitology* 163, 229-234.

- Mommer L., van Ruijven J., de Caluwe H., Smit-Tiekstra A.E., Wagemaker C.A.M., Ouborg N.J., Bögemann G.M., van der Weerden G.M., Berendse F. and de Kroon H. (2010) Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. *Journal of Ecology* 98, 1117-1127.
- Morin C., Bélanger G., Tremblay G.F., Bertrand A., Castonguay Y., Drapeau R., Michaud R., Berthiaume R. and Allard G. (2011) Diurnal variations of nonstructural carbohydrates and nutritive value in alfalfa. *Crop Science* 51, 1297-1306.
- Mortensen M.C., Schuman G.E. and Ingram L.J. (2004) Carbon sequestration in rangelands interseeded with yellow-flowering alfalfa (*Medicago sativa* ssp. *falcata*). *Environmental Management* 33, 475-481.
- Mueller-Harvey I. (2006) Unravelling the conundrum of tannins in animal nutrition and health. *Journal of the Science of Food and Agriculture* 86, 2010-2037.
- Naem S. and Li S. (1997) Biodiversity enhances ecosystem reliability. *Nature* 390, 507-509.
- Nesheim L. and Boller B.C. (1991) Nitrogen fixation by white clover when competing with grasses at moderately low temperatures. *Plant and Soil* 133, 47-56.
- Niderkorn V., Mueller-Harvey I., Le Morvan A. and Aufrère J. (2012) Synergistic effects of mixing cocksfoot and sainfoin on *in vitro* rumen fermentation. Role of condensed tannins. *Animal Feed Science and Technology* 178, 48-56.
- Novobilský A., Mueller-Harvey I. and Thamsborg S.M. (2011) Condensed tannins act against cattle nematodes. *Veterinary Parasitology* 182, 213-220.
- Nyfelner D. (2009) *Productivity and nitrogen utilization in productive agricultural grassland: effects of species combinations, species proportions and nitrogen fertilization*, PhD. Thesis ETH Zurich No. 18219, 179 pp.
- Nyfelner D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Lüscher A. (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology* 46, 683-691.
- Nyfelner D., Huguenin-Elie O., Suter M., Frossard E. and Lüscher A. (2011) Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems and Environment* 140, 155-163.
- Oberson A., Frossard E., Bühlmann C., Mayer J., Mäder P. and Lüscher A. (2013) Nitrogen fixation and transfer in grass-clover leys under organic and conventional cropping systems. *Plant and Soil* doi: 10.1007/s11104-013-1666-4.
- Oelmann Y., Buchmann N., Gleixner G., Habekost M., Roscher C., Rosenkranz S., Schulze E.D., Steinbeiss S., Temperton V.M., Weigelt A., Weisser W.W. and Wilcke W. (2011) Plant diversity effects on aboveground and belowground N pools in temperate grassland ecosystems: Development in the first 5 years after establishment. *Global Biogeochemical Cycles* 25, GB2014, doi:10.1029/2010GB003869.
- Orr R.J., Parson A.J., Penning P.D. and Treacher T.T. (1990) Sward composition, animal performance and the potential production of grass/white clover swards continuously stocked with sheep. *Grass and Forage Science* 45, 325-336.
- Palmberg C., Scherer-Lorenzen M., Jumpponen A., Carlsson G., Huss-Danell K. and Högberg P. (2005) Inorganic soil nitrogen under grassland plant communities of different species composition and diversity. *Oikos* 110, 271-282.
- Pelletier S., Tremblay G.F., Bélanger G., Bertrand A., Castonguay Y., Pageau D. and Drapeau R. (2010) Forage nonstructural carbohydrates and nutritive value as affected by time of cutting and species. *Agronomy Journal* 102, 1388-1398.
- Pellikaan W.F., Stringano E., Leenaars J., Bongers D.J.G.M., van Laar-van Schuppen S., Plant J. and Mueller-Harvey I. (2011) Evaluating the effects of tannins on the extent and rate of *in vitro* measured gas and methane production using the Automated Pressure Evaluation System (APES). *Animal Feed Science and Technology* 166-167, 377-390.
- Peyraud J.L. (1993) Comparaison de la digestion du trèfle blanc et des graminées prairiales chez la vache laitière. *Fourrages* 135, 465-473.
- Peyraud J.L., Le Gall A. and Lüscher A. (2009) Potential food production from forage legume-based-systems in Europe: an overview. *Irish Journal of Agricultural and Food Research* 48, 115-135.
- Peyraud J.L., Delaby L., Dourmad J.Y., Faverdin P., Morvan T. and Vertes F. (2012) Les systèmes de polyculture-élevage pour bien valoriser l'azote. *Innovations Agronomiques* 22, 45-69.
- Pflimlin A., Arnaud J.D., Gautier D. and Le Gall A. (2003) Les légumineuses fourragères, une voie pour concilier autonomie en protéines et préservation de l'environnement. *Fourrages* 174, 183-203.
- Phillips C.J.C. and James N.L. (1998) The effects of including white clover in perennial ryegrass pastures and the height of mixed swards on the milk production, pasture selection and ingestive behaviour of dairy cows. *Animal Science* 67, 195-202.
- Piano E. and Pecetti L. (2010) Minor Legume Species. In: Boller B., Posselt U.K. and Veronesi F. (eds) *Fodder Crops and Amenity Grasses, Handbook of plant breeding*, Vol. 5. Springer, New York, USA, pp. 477-500.



- Pirhofer-Walzl K., Rasmussen J., Høgh-Jensen H., Eriksen J., Sjøgaard K. and Rasmussen J. (2012) Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant and Soil* 350, 71-84.
- Provenza F.D. and Villalba J.J. (2010) The role of natural plant products in modulating the immune system: an adaptable approach for combating disease in grazing animals. *Small Ruminant Research* 89, 131-139.
- Rasmussen J., Sjøgaard K., Pirhofer-Walzl K. and Eriksen J. (2012) N<sub>2</sub>-fixation and residual N effect of four legume species and four companion grass species. *European Journal of Agronomy* 36, 66-74.
- Ribeiro-Filho H.M.N., Delagarde R. and Peyraud J.L. (2003) Inclusion of white clover in strip-grazed perennial ryegrass swards: herbage intake and milk yield of dairy cows at different ages of sward regrowth. *Animal Science* 77, 499-510.
- Ribeiro-Filho H.M.N., Delagarde R. and Peyraud, J.L. (2005) Herbage intake and milk yield of dairy cows grazing perennial ryegrass swards or white-clover/perennial rye grass swards at low- and medium-herbage allowances. *Animal Feed Science and Technology* 119, 13-27.
- Rochette P. and Janzen H.H. (2005) Towards a revised coefficient for estimating N<sub>2</sub>O emissions from legumes. *Nutrient Cycling in Agroecosystems* 73, 171-179.
- Rochon J.J., Doyle C.J., Greef J.M., Hopkins A., Molle G., Sitzia M., Scholefield D. and Smith C.J. (2004) Grazing legumes in Europe: a review of their status, management, benefits, research needs and future prospects. *Grass and Forage Science* 59, 197-214.
- Rockström J., Steffen W., Noone K., Persson Å., Chapin F.S., Lambin E.F., Lenton T.M., Scheffer M., Folke C., Schellnhuber H.J., Nykvist B., de Wit C.A., Hughes T., van der Leeuw S., Rodhe H., Sörlin S., Snyder P.K., Costanza R., Svedin U., Falkenmark M., Karlberg L., Corell R.W., Fabry V.J., Hansen J., Walker B., Liverman D., Richardson K., Crutzen P. and Foley J.A. (2009). A safe operating space for humanity. *Nature* 461, 472-475.
- Roscher C., Temperton V.M., Scherer-Lorenzen M., Schmitz M., Schumacher J., Schmid B., Buchmann N., Weisser W.W. and Schulze E.D. (2005) Overyielding in experimental grassland communities – irrespective of species pool or spatial scale. *Ecology Letters* 8, 419-429.
- Roscher C., Thein S., Schmid B. and Scherer-Lorenzen M. (2008) Complementary nitrogen use among potentially dominant species in a biodiversity experiment varies between two years. *Journal of Ecology* 96, 477-488.
- Roscher C., Thein S., Weigelt A., Temperton V.M., Buchmann N. and Schulze E.D. (2011) N<sub>2</sub> fixation and performance of 12 legume species in a 6-year grassland biodiversity experiment. *Plant and Soil* 341, 333-348.
- Roughley R.J. and Dart P.J. (1970) Growth of *Trifolium subterraneum* L. selected for sparse and abundant nodulation as affected by root temperature and Rhizobium strain. *Journal of Experimental Botany*, 21, 776-786.
- Rouillé B., Lamy J.M. and Brunschwig P. (2010) Trois formes de consommation de la luzerne pour les vaches laitières. *Rencontres Recherches Ruminants* 17, 329.
- Russelle M.P., Lamb J.F.S., Montgomery B.R., Elsenheimer D.W., Miller B.S. and Vance C.P. (2001). Alfalfa rapidly remediates excess inorganic nitrogen at a fertilizer spill site. *Journal of Environmental Quality* 30, 30-36.
- Ruz-Jerez B.E., White R.E. and Ball P.R. (1994) Long-term measurement of denitrification in three contrasting pastures grazed by sheep. *Soil Biology and Biochemistry* 26, 29-39.
- Saratsis A., Regos I., Tzanidakis N., Voutzourakis N., Stefanakis A., Treuter D., Joachim A. and Sotiraki S. (2012) *In vivo* and *in vitro* efficacy of sainfoin (*Onobrychis vicifolia*) against *Eimeria* spp. in lambs. *Veterinary Parasitology* 188, 1-9.
- Scherer-Lorenzen M., Palmborg C., Prinz A. and Schulze E.D. (2003) The role of plant diversity and composition for nitrate leaching in grasslands. *Ecology* 84, 1539-1552.
- Schils R.L.M. (1994) Nitrate losses from grazed grass and grass/clover pastures on clay soil. *Meststoffen* 1994, 78-84.
- Schils R.L.M., Verhagen A., Aarts H.F.M. and Šebek, L.B.J. (2005) A farm level approach to define successful mitigation strategies for GHG emissions from ruminant livestock systems. *Nutrient Cycling in Agroecosystems* 71, 163-175.
- Schmid B., Hector A., Saha P. and Loreau M. (2008) Biodiversity effects and transgressive overyielding. *Journal of Plant Ecology* 1, 95-102.
- Scholefield D., Lockyer D.R., Whitehead D.C. and Tyson K.C. (1991) A model to predict transformations and losses of nitrogen in UK pastures grazed by beef cattle. *Plant and Soil* 132, 165-177.
- Schulze E., Luysaert S., Ciais P., Freibauer A., Janssens I.A., Soussana J.F., Smith P., Grace J., Levin I., Thiruchittampalam B., Heimann M., Dolman A.J., Valentini R., Bousquet P., Peylin P., Peters W., Rödenbeck C., Etiope G., Vuichard N., Wattenbach M., Nabuurs G.J., Poussi Z., Nieschulze J. and Gash J.H. (2009) Importance of methane and nitrous oxide for Europe's terrestrial greenhouse-gas balance. *Nature Geoscience* 2, 842-850.
- Schwank O., Blum H. and Nösberger J. (1986) The influence of irradiance distribution on the growth of white clover (*Trifolium repens* L.) in differently managed canopies of permanent grassland. *Annals of Botany* 57, 273-281.

- Scioneaux A.N, Schmidt M.A., Moore M.A., Lindroth R.L., Wooley S.C. and Hagerman A.E. (2011) Qualitative variation in proanthocyanidin composition of *Populus* species and hybrids: genetics is the key. *Journal of Chemical Ecology* 37, 57-70.
- Sergeeva A.G. (1955) The effect of lucerne and sainfoin on the water-stable structure of soils under irrigation. *Pocvovedenie* 12, 35-42.
- Sölter U., Hopkins A., Sitzia M., Goby J.P. and Greef J.M. (2007) Seasonal changes in herbage mass and nutritive value of a range of grazed legume swards under Mediterranean and cool temperate conditions. *Grass and Forage Science* 62, 372-388.
- Soussana J.F. and Hartwig U.A. (1996) The effects of elevated CO<sub>2</sub> on symbiotic N<sub>2</sub> fixation: a link between the carbon and nitrogen cycles in grassland ecosystems. *Plant and Soil* 187, 321-332.
- Soussana J.F. and Lüscher A. (2007) Temperate grasslands and global atmospheric change: a review. *Grass and Forage Science* 62, 127-134.
- Soussana J.F. and Tallec T. (2010) Can we understand and predict the regulation of biological N<sub>2</sub> fixation in grassland ecosystems? *Nutrient Cycling in Agroecosystems* 88, 197-213.
- Soussana J.F., Minchin F.R., Macduff J.H., Raistrick N., Abberton M.T., and Michaelson-Yeates T.P.T. (2002) A simple model of feedback regulation for nitrate uptake and N<sub>2</sub> fixation in contrasting phenotypes of white clover. *Annals of Botany* 90, 139-147.
- Soussana J.F., Loiseau P., Vuichard N., Ceschia E., Balesdent J., Chevallier T. and Arrouays D. (2004) Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use and Management* 20, 219-230.
- Soussana J.F., Tallec T. and Blanfort V. (2010) Mitigating the greenhouse gas balance of ruminant production systems through carbon sequestration in grasslands. *Animal* 4, 334-350.
- Sparrow S. D., Cochran V. L. and Sparrow E. B. (1995) Dinitrogen fixation by seven legume crops in Alaska. *Agronomy Journal* 87, 34-41.
- Spehn E.M., Scherer-Lorenzen M., Schmid B., Hector A., Caldeira M.C., Dimitrakopoulos P.G., Finn J.A., Jumpponen A., O'Donovan G., Pereira J.S., Schulze E.D., Troumbis A.Y. and Körner C. (2002) The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. *Oikos* 98, 205-218.
- Speijers M.H.M., Fraser M.D., Theobald V.J. and Haresign W. (2004) The effects of grazing forage legumes on the performance of finishing lambs. *The Journal of Agricultural Science* 142, 483-493.
- Steg A., Van Straalen W.M., Hindle V.A., Wensink W.A., Dooper F.M.H. and Schils R.L.M. (1994) Rumen degradation and intestinal digestion of grass and clover at two maturity levels during the season in dairy cows. *Grass and Forage Science* 49, 378-390.
- Stoate C., Báldi A., Beja P., Boatman N.D., Herzog M., van Doorn A., de Snoo G.R., Rakosy L. and Ramwell C. (2009) Ecological impacts of early 21st century agricultural change in Europe – A review. *Journal of Environmental Management* 91, 22-46.
- Stringano E., Hayot Carbonero C., Smith L.M.J., Brown R.H. and Mueller-Harvey I. (2012) Proanthocyanidin diversity in the EU 'HealthyHay' sainfoin (*Onobrychis vicifolia*) germplasm collection. *Phytochemistry* 77, 197-208.
- Sturludóttir E., Brophy C., Bélanger G., Gustavsson A.M., Jørgensen M., Lunnan T. and Helgadóttir Á. (2013) Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. *Grass and Forage Science* doi: 10.1111/gfs.12037.
- Suter D., Briner H.U. and Lüscher A. (2007) Effect of *Lolium perenne* L. variety on the botanical composition of grass-clover mixtures. *Grassland Science in Europe* 12, 417-420.
- Suter D., Huguenin-Elie O., Nyfeler D. and Lüscher A. (2010) Agronomically improved grass-legume mixtures: higher dry matter yields and more persistent legume proportions. *Grassland Science in Europe* 15, 761-763.
- Suter D., Rosenberg E., Mosimann E. and Frick R. (2012) Standardmischungen für den Futterbau: Revision 2013-2016. *Agrarforschung Schweiz* 3, 1-12.
- Tallec T., Diquélou S., Avice J.C., Lesuffleur F., Lemauviel-Lavenant S., Cliquet J.B. and Ourry A. (2009) Availability of N and S affect nutrient acquisition efficiencies differently by *Trifolium repens* and *Lolium perenne* when grown in monoculture or in mixture. *Environmental and Experimental Botany* 66, 309-316.
- Tamminga S., Bannink A., Dijkstra J. and Zom R. (2007) Feeding strategies to reduce methane loss in cattle. *Animal Science Group, Wageningen University, February 2007, Report 34, 44p.* Available at: <http://www2.asg.wur.nl/NR/rdonlyres/F81D8745-6596-4296-A292-8553950E2B98/42707/34.pdf> (accessed on 6 February 2013).
- Tavendale M.H., Meagher L.P., Pacheco D., Walker N., Attwood G.T. and Sivakumaran S. (2005) Methane production from *in vitro* rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. *Animal Feed Science and Technology* 123-124, 403-419.

- Temperton V.M., Mwangi P.N., Scherer-Lorenzen M., Schmid B. and Buchmann N. (2007) Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. *Oecologia* 151, 190-205.
- Theodoridou K., Aufrère J., Andueza D., Le Morvan A., Picard F., Stringano E., Pourrat J., Mueller-Harvey I. and Baumont R. (2011) Effect of plant development during first and second growth cycle on chemical composition, condensed tannins and nutritive value of three sainfoin (*Onobrychis viciifolia*) varieties and lucerne. *Grass and Forage Science* 66, 402-414.
- Thomas R.J. (1992) The role of the legume in the nitrogen cycle of productive and sustainable pastures. *Grass and Forage Science* 47, 133-142.
- Thornton P.K. (2010) Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365, 2853-2867.
- Tibe O., Pernthaner A., Sutherland I., Lesperance L. and Harding D.R.K. (2012) Condensed tannins from Botswanan forage plants are effective priming agents of  $\gamma\delta$  T cells in ruminants. *Veterinary Immunology and Immunopathology* 146, 237-244.
- Tilman D. (1999) The ecological consequences of changes in biodiversity: A search for general principles. *Ecology* 80, 1455-1474.
- Tremblay G.F., Berthiaume R., Bélanger G., Bertrand A., Castonguay Y., Michaud R., Pelletier S., Brito A., Lafrenière C., Drapeau R. and Allard G. (2011) Nonstructural carbohydrates: How to increase their concentration in forages and how does it affect animal performance? In: Proceedings of the 47<sup>th</sup> Eastern Nutrition Conference, Montreal, Quebec, CA, pp. 219-243.
- Trenbath B.R. (1974) Biomass productivity of mixtures. *Advances in Agronomy* 26, 177-210.
- Ulyatt M.J. (1970) Evaluation of pasture quality under New Zealand conditions. *Proceedings of the New Zealand Grassland Association*, 32, 61-68.
- Undersander D., McCalsin M., Shaeffer C., Whalen D., Miller D., Putnam D. and Orloff S. (2009) Low lignin alfalfa: Redefining the yield/quality tradeoff. Available at: <http://alfalfa.ucdavis.edu/symposium/2009/> (accessed on 6 February 2013).
- Unkovich M.J., Baldock J. and Peoples M.B. (2010) Prospects and problems of simple linear models for estimating symbiotic N<sub>2</sub> fixation by crop and pasture legumes. *Plant and Soil* 329, 75-89.
- Van Dorland H.A., Wettstein H.R., Leunenberger H. and Kreuzer M. (2007) Effect of supplementation of fresh and ensiled clovers to ryegrass on nitrogen loss and methane emissions in dairy cows. *Livestock Science* 111, 57-69.
- Van Ruijven J. and Berendse F. (2003) Positive effects of plant species diversity on productivity in the absence of legumes. *Ecology Letters* 6, 170-175.
- Veronesi F., Brummer E.C. and Huyghe C. (2010) Alfalfa. In: Boller B., Posselt U.K. and Veronesi F. (eds) *Fodder Crops and Amenity Grasses, Handbook of plant breeding*, Vol. 5. Springer, New York, USA, pp. 395-438.
- Vertès F., Simon J.C., Le Corre L. and Decau M.L. (1997) Nitrogen flows in grazed pastures. II. Study of flows and their effects on leaching. *Fourrages* 151, 263-280.
- Vörösmarty C.J., McIntyre P.B., Gessner M.O., Dudgeon D., Prusevich A., Green P., Glidden S., Bunn S.E., Sullivan C.A., Reidy Liermann C. and Davies P.M. (2010) Global threats to human water security and river biodiversity. *Nature* 467, 555-561.
- Waghorn G.C. (2008) Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production – Progress and challenges. *Animal Feed Science and Technology* 147, 116-139.
- Waghorn G.C. and Hegarty R.S. (2011) Lowering ruminant methane emissions through improved feed conversion efficiency. *Animal Feed Science and Technology* 166-167, 291-301.
- Waghorn G.C., Shelton I.D. and Thomas V.J. (1989) Particle breakdown and rumen digestion of fresh ryegrass (*Lolium perenne* L.) and lucerne (*Medicago sativa* L.) fed to cows during a restricted feeding period. *British Journal of Nutrition* 61, 409-423.
- Waghorn G.C., Douglas G.B., Niezen J.H., McNabb W.C. and Foote A.G. (1998) Forages with condensed tannins – their management and nutritive value for ruminants. *Proceedings of the New Zealand Grassland Association* 60, 89-98.
- Waghorn G.C., Woodward S.L., Tavendale M. and Clark D.A. (2006) Inconsistencies in rumen methane production – effects of forage composition and animal genotype. *International Congress Series* 1293, 115-118.
- Wang Y., Barbieri L.R., Berg B.P. and McAllister T.A. (2007) Effects of mixing sainfoin with alfalfa on ensiling, ruminal fermentation and total tract digestion of silage. *Animal Feed Science and Technology* 135, 296-314.
- Wang Y., Majak W. and McAllister T.A. (2012) Frothy bloat in ruminants: cause, occurrence, and mitigation strategies. *Animal Feed Science and Technology* 172, 103-114.

- Weightman R.M., Cottrill B.R., Wiltshire J.J.J., Kindred D.R. and Sylvester-Bradley R. (2011) Opportunities for avoidance of land-use change through substitution of soya bean meal and cereals in European livestock diets with bioethanol coproducts. *Global Change Biology Bioenergy* 3, 158-170.
- Weiss P. and Raymond F. (1993) L'ensilage de trèfle violet pour l'engraissement des taurillons. *Fourrages* 134, 283-286.
- Wilkins R.J., Gibb M.J., Huckle C.A. and Clements A.J. (1994) Effect of supplementation on production by spring-calving dairy cows grazing swards of differing clover content. *Grass and Forage Science*, 49, 465-475.
- Woledge J. (1988) Competition between grass and clover in spring as affected by nitrogen-fertiliser. *Annals of Applied Biology* 112, 175-186.
- Woodward S.L., Waghorn G.C., Lassey K.R. and Laboyrie P.G. (2002) Does feeding sulla (*Hedysarum coronarium*) reduce methane emissions from dairy cows? *Proceedings of New Zealand Society of Animal Production* 62, 227-230.
- Woodward S.L., Waghorn G.C. and Laboyrie P.G. (2004) Condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) reduce methane emissions from dairy cows. *Proceedings of New Zealand Society of Animal Production* 64, 160-164.
- Woodward S.L., Waghorn G.C., Watkins K.A. and Bryant M.A. (2009) Feeding birdsfoot trefoil (*Lotus corniculatus*) reduces the environmental impacts of dairy farming. *Proceeding of the New Zealand Society of Animal Production* 69, 179-183.
- Yachi S. and Loreau M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America* 96, 1463-1468.
- Zanetti S., Hartwig U.A., van Kessel C., Lüscher A., Hebeisen T., Frehner M., Fischer B.U., Hendrey G.R., Blum H. and Nösberger J. (1997) Does nitrogen nutrition restrict the CO<sub>2</sub> response of fertile grassland lacking legumes? *Oecologia* 112, 17-25.