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Review Article

Potential of legume-based grassland–livestock systems in Europe: a review

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Abstract

European grassland-based livestock production systems face the challenge of producing more meat and milk to meet increasing world demands and to achieve this using fewer resources. Legumes offer great potential for achieving these objectives. 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 proportion of 30–50%. The resulting benefits include reduced dependence 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 the presence of bioactive secondary metabolites. In addition, legumes may offer an adaptation option to rising atmospheric CO_2 concentrations and 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 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 be expected that forage legumes will become more important in the future.

Keywords: yield, symbiotic N_2 fixation, forage quality, animal performance, greenhouse gas emission, nitrate leaching, animal health, climate change, energy, plant secondary metabolites, management, bloat, helminths, tannins.

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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 to respond and adapt to a changing climate. At the same time, grassland production faces competition from requirements for arable land, competition between food and feed production, requirements for bioenergy and the need to preserve biodiversity and maintain 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_2 fixation, (iii) mitigating and facilitating adaptation to climate change, as elevated atmospheric CO_2, 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), Legume Futures (www.legumefutures.eu), AnimalChange (www.animalchange.eu) and LegumePlus (www.legumeplus.eu). The objectives of MultiSward are 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

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biodiversity, and (ii) on optimizing their contribution to the economics, agronomy and nutritional inputs in order to achieve innovative and sustainable ruminant production systems. An important aspect is the investigation of the effects of multispecies mixtures, in which 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 Legume-Plus 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 also study the combined effects on nitrogen (N) 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 information on individual forage legumes as this information was compiled by Frame (2005) for more than thirty 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 most of the second half of the 20th century resulted in an abundant supply of cheap synthetic 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 have 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 contributions of public funding (in the Common Agricultural Policy) that are used to support European Agriculture are 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 when supported by high inputs of N-fertilizer (Frame, 1991; Daepp *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 forty-four 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 with the average monoculture. However, compared with the most productive monoculture, these mixtures showed a yield disadvantage of −12%. Transgressive overyielding, where mixtures outperform the best monoculture (Trenbath, 1974; Schmid *et al.*, 2008), occurred in only 12% of the experiments, and it took, on average, about 5 years to become evident. In an agronomic context, mixtures with transgressive overyielding are clearly preferred, as producers can select the highest-yielding species for monoculture cultivation and, thus, any mixture performance has to compete against this high benchmark.

A pan-European experiment on thirty-one sites in seventeen countries, which was carried out under the auspices of COST Action 852 ‘Quality legume-based forage systems for contrasting environments’ (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; Finn *et al.*, 2013). 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 3 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 average mixture 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%, when compared with the highest-yielding monoculture (Finn *et al.*, 2013). At the Swiss site, which tested red and white clovers, 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^{-1} year^{-1} fertilizer-N achieved the same yield as grass monocultures fertilized with 450 kg N ha^{-1} year^{-1} (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, a measure of the equality of species abundance in swards, had a highly significant effect on the yield and the diversity effect (the excess of mixture performance over that expected from average monoculture performances) (Kirwan *et al.*, 2007; Finn *et al.*, 2013; Sturludóttir *et al.*, 2013). At low values of evenness, a slight increase in mixture evenness resulted in a steep increase in 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 closely linked to legume proportions of 35–65%, the results of Finn *et al.* (2013) are in agreement with the recent finding of significant transgressive overyielding in mixtures over a wide range (30–80%) of clover proportions 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. As symbiotic N_2 fixation cannot explain the highly significant yield advantage of mixtures over legume monocultures (detailed results in Nyfeler *et al.*, 2009), access to atmospheric N_2 could not have been the only factor causing increased mixture yields. In diversity experiments, the positive interactions between N_2-fixing legumes and non-N_2-fixing plant species often contributed significantly more 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 also yield important diversity effects (Van Ruijven and Berendse, 2003; Roscher *et al.*, 2008). In the pan-European experiment, mixtures strongly benefitted from the combination of fast-establishing species 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 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 complementarity (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 (Suter *et al.*, 2010; Connolly *et al.*, 2013).

Legume yield is a main driver for large N inputs by symbiotic N_2 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 greatly increased 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 more than 160 teragrams (Tg) N year^{-1}, which clearly exceeds the amount supplied by biological N_2 fixation in natural systems (110 Tg N year^{-1}) (Gruber and Galloway, 2008; Herridge *et al.*, 2008). Substitution of industrial N-fertilizer by improved exploitation of N_2 fixation from symbiosis of legumes with *Rhizobium* bacteria would thus be an important contribution to more environmental-friendly and resource-efficient agricultural systems.

In grasslands, symbiotically fixed N_2 by legumes can range from 100 to 380 kg of N ha^{-1} year^{-1}, and exceptionally large amounts of >500 kg of N ha^{-1} year^{-1} have also been reported (Boller and Nösberger, 1987; Ledgard and Steele, 1992; Zanetti *et al.*, 1997; Carlsson and Huss-Danell, 2003). In addition, in mixed grass–legume systems, between 10 and 75 kg of N ha^{-1} year^{-1} are transferred from legumes to grass; the amount depends on the donor and the receiver plant species (Pirhofer-Walzl *et al.*, 2012). The control of symbiotic N_2 fixation operates through a series of ecophysiological triggers (Hartwig, 1998; Soussana *et al.*, 2002), with the amount of symbiotically fixed N_2 being tightly coupled to the gap between N demand (sink) and N availability (source) from mineral-N sources, at different 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 clovers, Nyfeler *et al.* (2011) observed stimulatory effects of the accompanying grasses on the symbiotic N_2 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) proportions of 40–60% clovers in the mixture were sufficient to attain the same amount of N from symbiosis as that in pure clover stands. This stimulatory effect fits well into the sink/source model of the regulation of symbiotic N_2 fixation. The activity of symbiotic N_2 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 competitive grass component taking up most of the mineral N that was available in the soil, while clover showed only very limited uptake (Nyfeler *et al.*, 2011). However, in clover-dominated swards (>60% of clovers), the activity of symbiotic N_2 fixation was down-regulated. This was due to the clover having adequate access to mineral-N sources because of low grass abundance (Nyfeler *et al.*, 2011) as well as a significantly lower N demand of the whole sward, as clover-dominated swards were less productive than swards with 30–60% of clover (Nyfeler *et al.*, 2009). The sensitivity of legume plants to down-regulate their symbiotic N_2 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) obtain most of their N (>80%) from symbiotic

N_2 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 generally 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 unfertilized or low-fertilized grassland experiments, high values of the proportion of N derived from symbiosis have also been measured for 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_2 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 N accumulation of white clover, but also to a marked reduction in the proportion of N derived from symbiosis. Thus, the authors concluded that the negative effects of low temperature on processes of N_2 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 N_2 fixation to N nutrition of white clover at low temperatures. Only few field studies have been published on symbiotic N_2 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,b) studied the significance of symbiotic N_2 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. badium* 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 legume species met most of their N requirements from symbiosis (59–90%). This suggests that symbiotic N_2 fixation is well adapted both to the climatic and acidic soil conditions (pH 5·6–4·1) of the sites investigated. Nevertheless, the amount of N derived from symbiosis decreased significantly with increasing altitude (from 18 to 1 kg N ha^{-1} $year^{-1}$) 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_2 fixation at the ecosystem level, have several critical implications for the exploitation of atmospheric N_2 in grassland systems. First, compared with clover monocultures, grass–clover mixtures have the potential to fix more N_2 from the atmosphere. Second, there is no trade-off between high productivity and high gains of symbiotically fixed N_2 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 the demand for 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 increase the risk of N losses to the environment. These unwanted N losses can be reduced or prevented, however, as long as the proportion of grasses in the sward is sufficient 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 many forages were summarized by INRA (2007). White clover, red clover and lucerne have high concentrations of crude protein (CP) and minerals, such as calcium, but they contain relatively low concentrations of water-soluble carbohydrates (WSC), compared with perennial ryegrass (*Lolium perenne* L.). 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 greatest for lucerne [5·54, 6·10 and 7·17 MJ kg DM^{-1} 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 are high when fed as fresh forage and are almost at the recommended level for optimal feeding of dairy cows (INRA, 2007) and higher than that recommended for low-producing cows. The high metabolizable protein content is maintained by hay making but it is reduced during ensiling.

Voluntary intake is basically measured on sheep fed *ad libitum* and expressed in g DM (dry matter) per kg of metabolic weight (INRA, 2007). Voluntary intake of legume forage is 10–15% greater than that of grasses of similar digestibility, and this is true whether forage legumes are fed as silage, hay or as fresh herbage (INRA, 2007). This difference is 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 2–3 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, using Hereford steers, Alder and Minson (1963) found that herbage intake was 15–20% higher with pure lucerne relative to that of pure cocksfoot. 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 the 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 decreased by 20 g kg^{-1} per week and voluntary DM intake by 0·2 kg d^{-1} 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 d^{-1} on a predominantly grass sward compared with 0·8 kg d^{-1} on mixed grass–clover swards. This makes mixed pasture swards much easier to manage than pure grass pastures, in terms of maintaining their nutritive value, and this greater flexibility makes mixed pastures particularly attractive for farmers. For example, it allows intervals between two successive grazing periods of more than 4–6 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 in legume–grass mixtures compared with monocultures was not accompanied by the reduction in herbage digestibility and crude protein concentration that is usually observed with increased DM yield. Low-lignin lucerne cultivars could provide another opportunity for further reducing the decline of nutritive value that occurs 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 that obtained from pure grass silages (Castle *et al.*, 1983; Dewhurst *et al.*, 2003). Chenais *et al.* (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^{-1}. The same applied to beef production, where feeding red clover resulted in identical growth rates to feeding maize silage, as long as the red clover silage was well preserved (Weiss and Raymond, 1993). It should be pointed out that legumes can be difficult to conserve, however, and 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 is at its maximum (Brito *et al.*, 2008; Pelletier *et al.*, 2010; Morin *et al.*, 2011). Plant breeding and gene manipulation might also be options for increasing the TNC in legume plants (Tremblay *et al.*, 2011).

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

As mixed white clover–grass pastures are usually managed with very low inputs of N fertilization, the biomass per hectare might be lower than from highly fertilized grass pastures at the same age of regrowth. Therefore, mixed pastures often have lower milk yields and liveweight gains per hectare than pure perennial ryegrass pastures, as their stocking rates are generally slightly lower 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 many farmers. Alternatively, more flexibility in utilization of mixed pastures allows 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 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^{-1} DM intake, and the amount of N that enters the duodenum is always less than N intake, averaging 75% of N intake for white clover compared with 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^{-1} milk on mixed white clover–perennial ryegrass pastures compared with perennial ryegrass pastures.

The WSC contained within the forage should be sufficient to balance the 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 through 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). Legume forages, however, 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. Scollan, Aberystwyth University, UK, *personal communication*). 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 that occurs during ensilage worsens the imbalance between degradable protein and energy in legume silages, and this leads to inefficient N utilization and high urinary-N excretion (Dewhurst *et al.*, 2003, 2009; Cohen *et al.*, 2006). 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 can complement maize silage in mixed diets (Chenais *et al.*, 1993; Rouillé *et al.*, 2010), as they can provide sources of both degradable protein and undegradable protein. They also offer some potential to substitute imported soya bean 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 reviews information on several other forage legumes that possess additional features and which may offer opportunities for ruminant nutrition and health, and for reducing the greenhouse gas emissions from ruminant agriculture. 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 legume species with such features. Sainfoin (*Onobrychis viciifolia* Scop.) holds particular promise for alkaline and drought-prone soils, which cover much of

Table 1 Advantages and disadvantages of bioactive legume species suitable for a range of European environments and soil conditions.

Characteristics	Legume species *Trifolium pratense* Red clover	 *Lotus corniculatus* Birdsfoot trefoil	 *Onobrychis viciifolia* Sainfoin
Latitude (°N)	35°–64°	35°–56°	35°–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 Waterlogging	Fair	Good	Poor
Drought tolerance	Medium-poor	Medium-poor	Good
Anthelmintic	No	Low	Yes
Antibloating	No	Yes	Yes
Oestrogenic	Yes	No	No
Bioactive constituents	Polyphenol oxidase	Tannins	Tannins

central and southern Europe (Sölter *et al.*, 2007). All legumes improve soil fertility and thus contribute to sustainability, but sainfoin has been reported to contribute 16 200 kg ha^{-1} of dry matter from fine roots, compared with 4200 kg ha^{-1} 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 relatively few cultivars of sainfoin or birdsfoot trefoil (*Lotus corniculatus* L.) 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 the leaves and stems of several forage legumes, such as birdsfoot trefoil, sainfoin, sulla (*Hedysarum coronarium* L.) sericea lespedeza (*Lespedeza cuneata* G. Don) and also in the flowers of *Trifolium* species (Waghorn *et al.*, 1998; Mueller-Harvey, 2006; Waghorn, 2008; Terrill *et al.*, 2012; Piluzza *et al.*, 2014). Total concentrations and compositions depend on accession/variety (Häring *et al.*, 2007; Azuhnwi *et al.*, 2011; Stringano *et al.*, 2012), season (Theodoridou *et al.*, 2011), plant organ (Häring *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 that contain condensed tannins. Although the biosynthesis of monomeric polyphenols and flavanols is now known, identifying the genes and enzymes involved in the synthesis of condensed tannins is still the subject of research (Dixon *et al.*, 2012; Brillouet *et al.*, 2013). 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), questions remain as to how amenable these traits are to improvement by plant breeding. Research will also be needed to develop screening tools that are suitable for breeding new legume varieties with optimized composition of condensed tannins (Reid *et al.*, 2013; Engström *et al.*, 2014).

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 reduce 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 some trefoil species/cultivars, particularly of big trefoil (*Lotus pedunculatus* Cav., var. Grasslands Maku), may be too 'potent' as ruminants cannot utilize its dietary protein fully, as evidenced by high faecal N contents (Waghorn *et al.*, 1998). In other cases, however, (e.g. birdsfoot trefoil, *Lotus corniculatus*, var. Grasslands Goldie, and in 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 that are present in sainfoin. Promising results have indeed 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.*, 2012).

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 N and slightly more faecal N, compared with other isonitrogenous diets. This is important because urinary urea is quickly converted to ammonia and nitrous oxide (N_2O), which has implications for environmental pollution, whereas faecal N 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 twofold among legumes and, together with other features, contributed to 20-fold differences in protein half-lives in a simulated, but micro-organism-free, rumen environment (a half-life of 19 h in sainfoin vs. 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; Lee *et al.*, 2013). 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 and also financial losses to farmers. It generally occurs when plants degrade too fast in the rumen; this produces a stable proteinaceous foam that traps fermentation gases, which can no longer be eructed by the animal (Wang *et al.*, 2012). However, plants containing condensed tannins, such as sainfoin, birdsfoot trefoil, crownvetch (*Coronilla varia* L.) and cicer milkvetch (*Astragalus cicer* L.), either as sole feeds 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 are parasites that cause diarrhoea in many animals, including cattle, sheep and goats, and can result in serious economic losses (D.S. Kommuru, T. Barker, S. Desai, J.M. Burke, A. Ramsay, I. Mueller-Harvey, J.E. Miller, J.A. Mosjidis, N. Kamisetti and T.H. Terrill, submitted). 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 also effective against flystrike in sheep, which occurs when sheep are affected by wet faeces. Flystrike 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*, rather than on veterinary drugs to control *Haemonchus contortus* infections (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; Martínez-Ortíz-de-Montellano *et al*., 2013). It is of particular interest that the anthelmintic bioactivity was still present or even enhanced after sainfoin was conserved as hay or 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 newborn 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; Azuhnwi *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_2, (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 contribute to reductions in 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; Palmborg *et al*., 2005). Loiseau *et al.* (2001) reported higher annual leaching losses of N from lysimeters when swards were sown with pure white clover (losses of 28–140 kg N ha^{-1}) than with pure perennial ryegrass (1–10 kg N ha^{-1}), but much higher values were reported for bare soils (84–149 kg N ha^{-1}). However, as long as the proportion 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. Indeed, under a mowing regime, Nyfeler (2009) found no increased risk of leaching of nitrate as long as the percentage of legumes in the mixture was below 60–80%, and this was combined with moderate N-fertilizer input. 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_3-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 by differences in stocking rates, as mixed pastures do not support stocking rates as high as those of fertilized grass pastures, and to a lesser extent by the down-regulation of symbiotic N_2 fixation under high mineral-N availability. At a similar stocking rate (3·3 cows ha^{-1}) and milk yield per hectare (13 200 kg milk ha^{-1}), Ledgard *et al.* (2009) reported similar leaching of N (30 kg N ha^{-1}) under mixed grass–clover pastures and pure grass fertilized with 160 kg N ha^{-1}, whereas N leaching increased to 60 kg N ha^{-1} for a more intensively fertilized grass pasture (207 kg N-fertilizer ha^{-1}, 15 500 kg milk ha^{-1}). Also, Vertès *et al.* (1997) found a 5–10% reduction of NO_3^- leaching under grass–clover, compared with 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, although leaching was recorded from grazed grass–legume plots on four sites in Europe, where ten legume species were evaluated over 3 years (Sullas *et al.*, 2012). On these sites, losses of N through leaching were low, with few differences between treatments, and they were well within legislative limits. Leaching losses were also lower under pastures with a lucerne–grass mixture than 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 ammonium-N in the soil (Jarvis and Barraclough, 1991).

Forage legumes can contribute to lower greenhouse gas emissions

Methane

Methane produced in the rumen is a large contributor to the greenhouse gas emissions of livestock systems (Tamminga *et al.*, 2007; Waghorn and Hegarty, 2011). Legumes can contribute to reducing ruminal methane production per unit of intake. Ruminants that are fed legume forages generally emitted less methane than grass-fed animals, per unit of feed intake (McCaughey *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 towards 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 antimethanogenic effect of condensed tannins above 20 g kg^{-1} 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 antimethanogenic 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 antimethanogenic 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_2 equivalents) in the form of 19 g N_2O (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_2O, which is a greenhouse gas that is *ca.* 300 times more potent than CO_2 (Kingston-Smith *et al.*, 2010). The process of denitrification is the most important source of N_2O from pasture-based 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_2O emissions are measured in grasslands immediately after N-fertilizer applications (Ineson *et al.*, 1998; Klumpp *et al.*, 2011). There are three reasons why N_2O emissions from legume-based grassland systems should be lower than from fertilized grass systems: (i) N is fixed symbiotically within the legume nodules and thus is not freely available in the soil in a reactive form, (ii) symbiotic N_2 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_2O emissions were largest in N-fertilized grass swards (19 site-years; 4·49 kg N_2O-N ha^{-1}) followed by pure legume stands (17 site-years; 0·79 and 1·99 kg N_2O-N ha^{-1} for white clover and lucerne, respectively) and mixed grass–clover swards (eight site-years; 0·54 kg N_2O-N ha^{-1}). Within the revised greenhouse gas guidelines (IPCC, 2006), symbiotic N_2 fixation has actually been removed as a direct source of N_2O. There are occasional observations of increased N_2O emissions by legumes (e.g. Virkajärvi *et al.*, 2010). There is, however, a lack of evidence of significant emissions arising from the fixation process itself (Rochette and Janzen, 2005). These authors concluded that the N_2O emissions induced by the growth of legume crops/forages may be estimated solely as a function of the above-ground and below-ground N inputs from crop/forage residue during pasture renewal. Emissions of N_2O 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).

Carbon dioxide

Industrial production of each kg of inorganic N emits 2·25 kg of CO_2. Legumes offer a big advantage because the entire C needed for symbiotic N_2 fixation comes directly from the atmosphere via photosynthesis and, thus, they are considered to be 'greenhouse gas neutral'. A further option to mitigate CO_2 emission (and climate change) is by 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^{-1} 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_2 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).

Greenhouse gases from dairy farms

At the level of the whole livestock system, Ledgard *et al.* (2009) and Basset-Mens *et al.* (2009) showed using life-cycle analysis that greenhouse gas emissions decreased by 1·15–1·00 kg eq-CO_2 kg^{-1} milk with mixed grass–clover pastures compared with pure grass pastures because of the reduction of N_2O 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 pastures of grass–white clover swards, which are stocked all year round and receive an annual N-fertilizer input of 100 kg ha^{-1}, and less than 10% of the feed requirement of the cows is provided by supplementary feed. They showed that the total emission per 1 kg milk is 30–80% lower from the 'New Zealand system'. Greenhouse gas emissions are high from intensive European dairy farms based on predominantly grass pastures; the contribution of methane is reduced in proportion, and CO_2 emissions were also 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 also because of effluent management. Schils *et al.* (2005) compared the total emissions from dairy systems in the Netherlands, which were either fertilized swards of ryegrass or grass–clover (i.e. inputs of 208 and 17 kg mineral-N ha^{-1} $year^{-1}$): greenhouse gas emissions per kg of milk were 20% lower for grass–clover pasture-based systems.

Forage legumes lower the need for non-renewable energy inputs

The introduction of legumes reduces non-renewable energy consumption in livestock farms because they use atmospheric N, and 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 *et al.*, 1999). Studies estimated that under French conditions, 0·17 MJ of energy is required to produce 1 MJ of net energy with ryegrass fertilized at 150 kg N ha^{-1}, 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^{-1} milk for intensive dairy farms in the Netherlands to 4·0 MJ kg^{-1} milk for French farms using maize silage and fertilized grasses and to 3·1 and 1·4 MJ kg^{-1} 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 fertilizer-N on pure ryegrass pastures, in comparison with the lower fertilizer-N use 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_2 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. Under field conditions, however, N is the major limiting factor in the yield response of grasslands to elevated CO_2. Thus, elevated atmospheric CO_2 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 to 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 conditions, where key nutrients were not limiting growth and N_2-fixation (Almeida *et al.*, 2000; Lüscher *et al.*, 2004; Watanabe *et al.*, 2013), legumes benefit more from elevated atmospheric CO_2 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_2 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_2 concentrations was derived solely from increased activity of symbiotic N_2 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 therefore 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 also 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 with 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) and LegumePlus (www.legumeplus.eu).

Technology needs for achieving a more stable abundance of legumes in the sward

In conclusion, grass–clover mixtures with the legumes present in proportions of 30 to 50% seem to be an optimal system: they yield high amounts of N from symbiosis, they generate high forage yields of high nutritive value, which in turn lead to 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_2 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_2 fixation is energetically costly and N_2 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 the 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_2 enrichment (FACE) experiment (Hebeisen *et al.*, 1997; Zanetti *et al.*, 1997). Averaged over the first 3 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 proportions 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, in addition to 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 improved several times during the last few decades, based on experimental results and observations on farms. Over the 3 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 resultant 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 multifactorial opportunity for optimization.

Conclusions

As components 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. Their advantages are most pronounced in mixed swards with 30–50% of legumes. These advantages are as follows: (i) increased forage production; (ii) ‘greenhouse gas neutral’ and ‘energy neutral’ N input into grasslands via symbiotic N_2 fixation; (iii) support of non-N_2-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, leading to (v) higher livestock performance. In addition, bioactive plant secondary metabolites of legumes can (vi) enhance the efficiency of protein digestion by ruminants and (vii) benefit animal health through reducing the need for 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_2 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 exploit more fully the opportunities they offer. The most important areas for research are as follows: (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.

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