ANIMALCHANGE

SEVENTH FRAMEWORK PROGRAMME

THEME 2: FOOD, AGRICULTURE AND FISHERIES, AND BIOTECHNOLOGIES

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DELIVERABLE 7.3

Quantification of the effect of legume proportion in the sward on yield advantage and options to keep stable legume proportions (over climatic zones relevant for livestock production)

Abstract: Grassland systems using mixed grass-legume swards have higher productivity and less environmental impact than their respective monocultures. The positive effect of legumes on yield is most pronounced with a mixture legume proportion of 30-60%.

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1. Summary

Grassland-based livestock production faces global challenges to meet the growing demand for meat and milk through increased production. This goal, however, has to be achieved using fewer resources in a more sustainable way than so far. Legumes offer great potential for coping with such challenges. Legumes have access to atmospheric nitrogen through symbiotic N_2 fixation, and they exhibit numerous features that impact positively on the soil-plant-animal-atmosphere system. Their positive contribution to grassland systems is most pronounced in mixed swards with a legume proportion of 30-60%. The resulting benefits include higher productivity and increased protein self-sufficiency, lower costs of production, a reduced dependency on both fossil energy and inorganic N fertilizer, and lower quantities of harmful emissions to the environment. Due to increased productivity and reduced emissions, both per area cropped, these benefits are evident not only at the functional unit of managed land area but also at the unit of the final product. This makes legumes to a key option for sustainable intensification of agriculture.

However, legumes suffer from some limitations, one of which is a reduction in their proportion in mixed swards over time. In temperate regions of Europe, stable legume proportions in swards can be achieved by the following management practices: sowing more complex mixtures that include several grass and legume species and/or adapted cultivars, reducing N fertilizer input, and adjusting cutting and grazing rates. These practices need not necessarily reduce production levels. Growth restrictions of legumes due to low winter temperatures and/or limited water availability should be counteracted by selecting legume species and cultivars adapted to these environmental conditions. In conclusion, the promotion and development of legume-based grassland systems undoubtedly constitutes a highly relevant support for more environmentally sustainable and commercially competitive grassland-livestock systems.

2. Legume-grass swards: a key to sustainable intensification of agriculture

Under fertile agricultural conditions, monocultures of selected, highly productive grass species give high forage yields that are dependent on high inputs of fertilizer nitrogen (N) (Frame 1991; Daepp *et al.* 2001). Although there is strong demand to increase global food production, maximizing high forage yield in livestock systems through the use of industrially-produced fertilizers has recently been challenged through several lines of evidence. First, substantial N losses in highly fertilized grass monocultures due to nitrate (NO_3) leaching (Thomas 1992; Hooda *et al.* 1998; Ledgard *et al.* 2009; Peyraud *et al.* 2009) pose a threat to human health as leachates can reach groundwater resources used for drinking water (Squillace *et al.* 2002). Second, greenhouse gas emissions of 3.3 and 8.6 kg CO_2-equivalents are calculated per kg of urea-N and ammonium nitrate-N, respectively, for production and transport to the regional storehouse (global warming potential of over 100 years according to Christensen *et al.* 2007; database ecoinvent data v2.2 2010). Third, each kg of inorganic N produced in the industrial Haber-Bosch process consumes large amounts of energy (Kaltschmitt and Reinhardt 1997; Kitani *et al.* 1999). It is axiomatic that current levels of crop and livestock production have to be maintained or even to be increased; however, the manifold deleterious side-effects of intensive N fertilizer application to natural ecosystems call for more sustainable production than so far (Guckert and Hay 2001;

Rockström *et al.* 2009). The need to foster sustainable, resource-efficient yet productive agricultural systems poses a new challenge not only to European countries but also to other regions worldwide.

The use of mixtures instead of monocultures in grassland systems can be expected to be a promising strategy to increase yield. Established theory suggests that 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). Indeed, many experiments in nutrient-poor grasslands have shown that biomass production was enhanced in species-rich communities, compared to the average yield of the respective monocultures (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). The presence of legumes in mixtures had generally a more pronounced positive effect on biomass yield than the presences of other functional groups (e.g. forbs, grasses) or species richness itself (Lambers *et al.* 2004; Thompson *et al.* 2005; Marquard *et al.* 2009). In a meta-analysis of 44 biodiversity experiments in which plant species richness was manipulated, Cardinale *et al.* (2007) found that mixtures, on average, outperformed monocultures by +77%. Yet, compared to 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 cases and it took about five years to become evident. In an agronomic context, however, 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.

The combination of grasses with legumes in agricultural systems offers great potential for maintaining not only high levels of production, but doing so in an environmentally sustainable manner. Legumes exhibit numerous features that impact positively on the soil-plant-animal-atmosphere system (Castle *et al.* 1983; Hooda *et al.* 1998; Dewhurst *et al.* 2009; Ledgard *et al.* 2009; Peyraud *et al.* 2009; Soussana *et al.* 2010; Jensen *et al.* 2012) and comprehensive research has established their positive contribution to grassland systems in terms of biomass yield (Kirwan *et al.* 2007; Nyfeler *et al.* 2009; Finn *et al.* 2013) and harvest quality (Frehner *et al.* 1997; Nyfeler *et al.* 2011). Regarding quality, forage from grass-legume mixtures leads to an improved balance of the protein-energy ratio and increased digestibility of animal diet compared to grass or legumes alone (Sleugh *et al.* 2000; Marshall *et al.* 2004; Baumont *et al.* 2008; Dewhurst *et al.* 2009). The resulting benefits are higher productivity and increased protein self-sufficiency, lower costs of production, a reduced dependency on fossil energy and mineral N fertilizers, and lower quantities of harmful emissions to the environment (greenhouse gases and nitrate).

3. Overyielding and transgressive overyielding in grass-legume mixtures

3.1 Overyielding

One of the most comprehensive agrodiversity experiments ever conducted involved 31 sites in 17 countries under the auspices of the COST Action 852, 'Quality legume-based forage systems for contrasting environments' (www.cost.eu/domains_actions/fa/Actions/852). The geographical range of sites spanned a gradient of climate from Atlantic to continental, and from temperate to arctic (see Annex 1 for a list of included sites). Under conditions typical of cutting regimes, it was tested whether higher yields can be achieved with grass-legume mixtures containing four species as compared to the respective monocultures (Kirwan *et al.* 2007; Nyfeler *et al.* 2009). The four species represented four functional groups, which were chosen so as to maximize beneficial interspecific interactions: a fast-establishing grass, a fast-establishing legume, a temporarily-persistent grass, and a temporarily-persistent legume. The grass-legume combinations were intended to extend the method of nitrogen acquisition, as legumes have access to the unlimited resource of atmospheric nitrogen through symbiotic N_2 fixation (Hartwig 1998; Soussana *et al.* 2002), while fast/persistent combinations were intended to maximize sward cover by varying patterns of species 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), meaning that legumes other than the widely investigated white and red clover were also included. Stands were established in two sowing densities and with greatly varying relative abundances of species, and received N fertilisers according to the general productivity level of sites. Across the three years of experimental duration, the yield of sown species (total yield excluding weed biomass) in mixtures was higher than the average monoculture in 99.7% of comparisons, with mixtures having, on average, 77% more yield than the average of monocultures (Fig. 1). Overyielding in harvest yield of mixtures appeared already in the first year (being 62%) and was maintained for at least three years (82% and 116% in second and third years, respectively) (Finn *et al.* 2013). It was most remarkable that overyielding occurred at *all* sites, which spanned a latitudinal range from 40°44'N (Sardinia, Italy) to 69°40'N (Tromsø, Norway), indicating that the relative benefit of yield was not restricted to highly productive sites (Fig. 1, to the left), but was equally apparent to the same relative degree at low yielding sites (Fig. 1, to the right), e.g. arctic or Mediterranean sites (Sturludóttir *et al.* 2013).

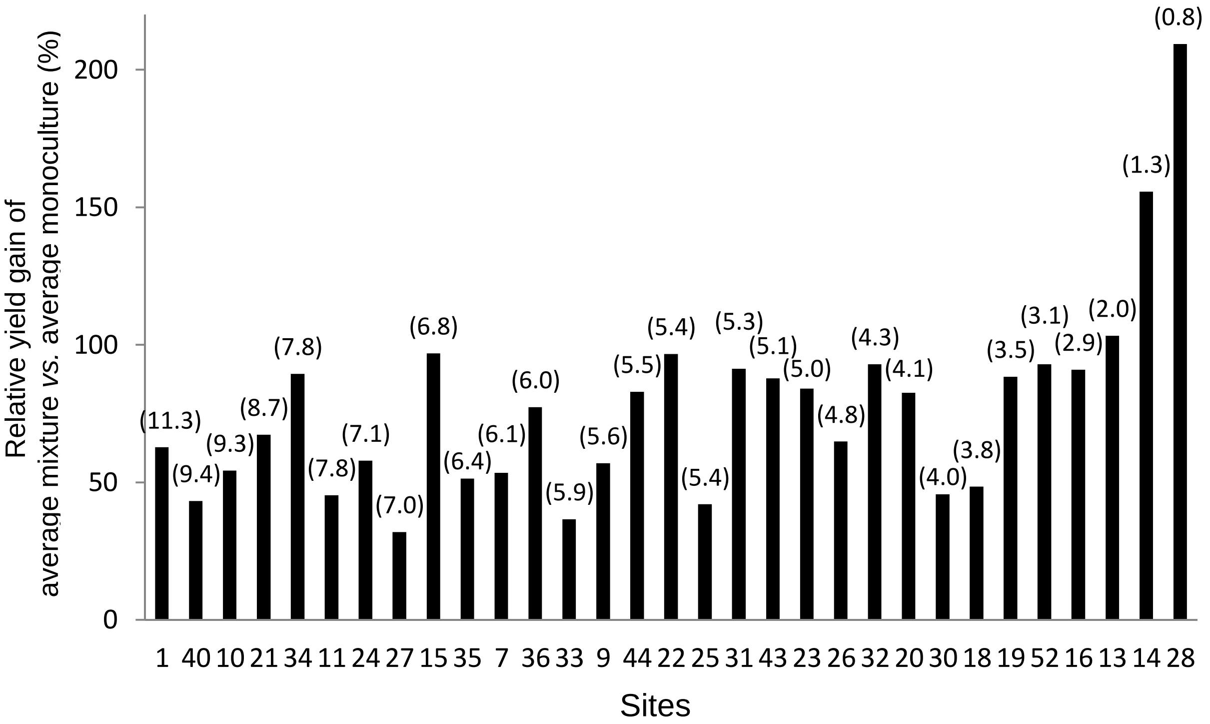

Fig. 1. Relative benefit of average mixture yield of sown species *versus* average yield of the respective monocultures over the whole experimental duration in %. Sites are arranged in order of decreasing means of monoculture yields (values above bars in t ha^{-1} year^{-1}), indicating that the mixture overyielding was not restricted to high yielding sites (to the left). Site names to numbers and further site information are given in Annex 1.

3.2 Transgressive overyielding

While overyielding (the degree to which mixture yield exceeds the *average* yield of monocultures) was prevalent in the pan-European COST 852 experiment, it was most remarkable that transgressive overyielding (TO) was evident at the majority of sites (mixtures outperform the best monoculture, Finn *et al.* 2013). Across all experimental years, the total annual yield (sown species plus weed biomass) of mixtures exceeded that of the best monoculture in about 60% of sites, with the benefit of mixtures being 7% (Fig. 2). However, if yield of sown species (excluding weed biomass) was considered, TO was significant at about 70% of sites, with a mixture benefit of 18%. This is a highly valuable result because it demonstrates that mixtures of agronomically relevant grass-legume species outperform even the best monoculture that is usually selected to achieve maximal yields (often a grass species in highly fertilized monoculture systems).

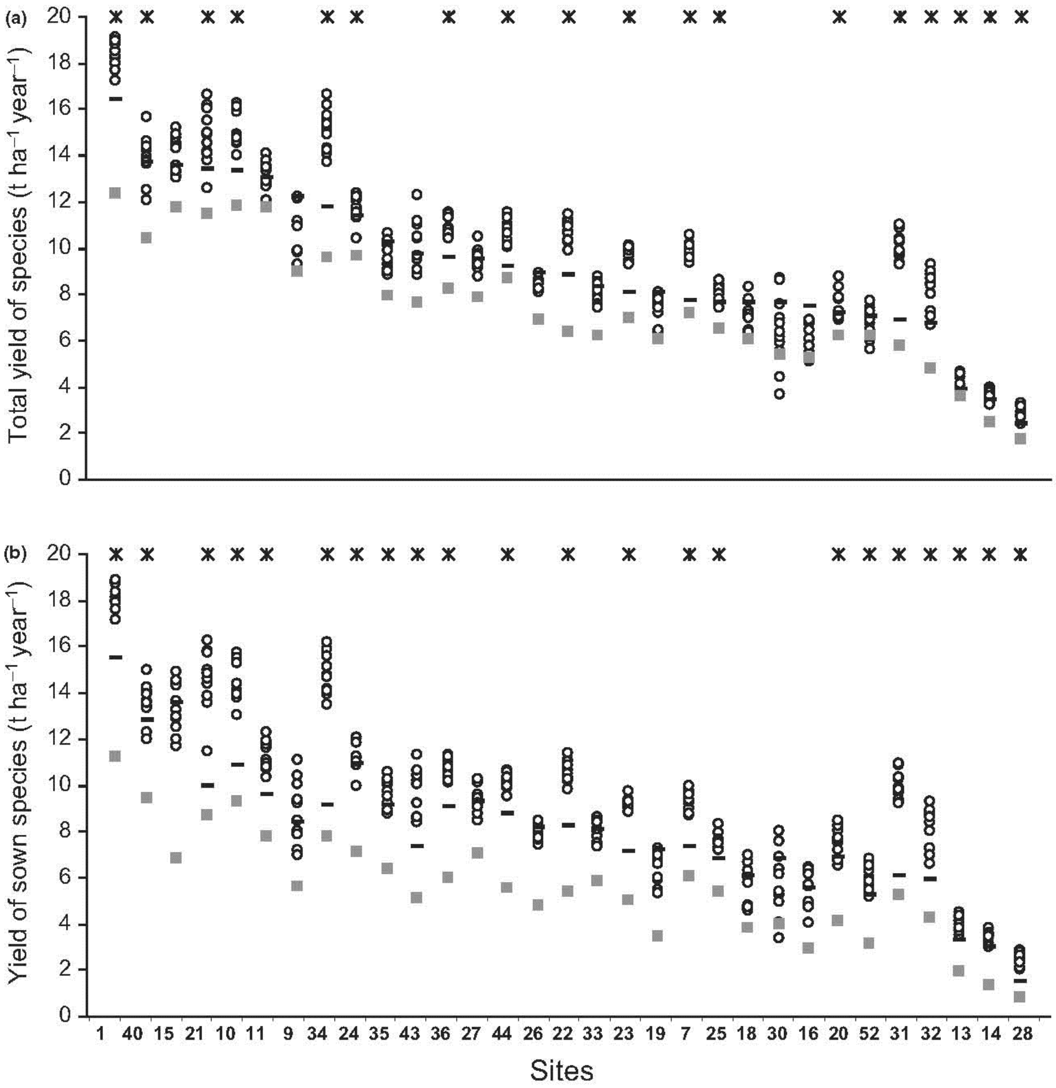

Fig. 2. Average annual yield (dry matter) over the whole experimental duration of (a) total yield and (b) yield of sown agronomic species only (excludes weeds) at each of 31 sites. For all communities, these data are averaged across seed density and across years per site. Sites are arranged in order of decreasing total yield of the best-performing monoculture. Open circles represent each of the 11 mixture communities that differed in their relative abundance at sowing; horizontal bars represent the yield of the best-performing monoculture; squares represent the mean monoculture performance. Significant transgressive overyielding is indicated by an asterisk (**ж**) over a site at the top of each panel. Note that not all of these sites had 3 years of yield data (see Table A1, Annex 1).

This figure is provided (with permission) from Finn *et al.* (2013). *Figure number changed and legend shortened.*

For example, at the Swiss site of the experiment, grass-clover mixtures fertilized with 50 or 150 kg of N ha^{-1} year^{-1} had maximal yields of 15.2 t and 16.1 t ha^{-1} year^{-1}, respectively, and thereby exceeded significantly the yields of the heavily fertilized (450 kg of N ha^{-1} year^{-1}) monocultures of the productive grass species *Lolium perenne* L. (perennial ryegrass, 12.0 t dry matter ha^{-1} year^{-1}) and *Dactylis glomerata* L. (cocksfoot, 13.4 t dry matter ha^{-1} year^{-1}, Nyfeler *et al.* 2009). With respect to all European sites, the high degree of TO appeared in the first year, persisted for at least three years and was not restricted to high yielding sites (Fig. 2) (Finn *et al.* 2013). Thus, similar to overyielding, TO was prevalent also at the low yielding sites, which became directly apparent when computing the ratio of mixture yield to the yield of the best performing monoculture (Fig. 3).

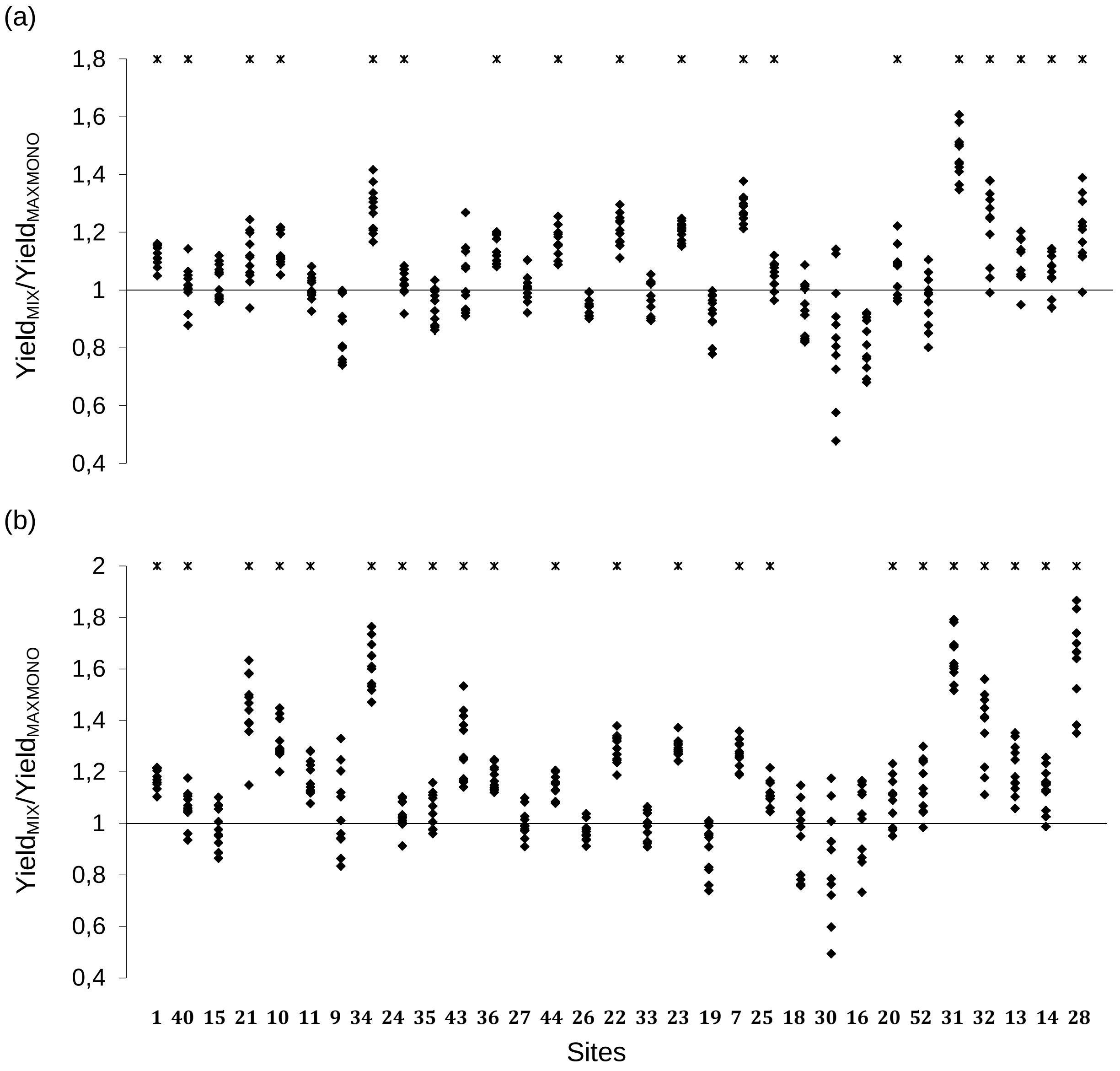

Fig. 3. Ratio of yield of each mixture community to yield of the best-performing monoculture (across years) at each site based on a) total yield and b) yield of sown species. Each point represents the sum of aboveground biomass over multiple years. Significant transgressive overyielding is indicated by an asterisk (**ж**) over a site at the top of each panel. Sites arranged in order of decreasing total yield of the best-performing monoculture (as in Fig. 2).

This figure is provided (with permission) from Finn *et al.* (2013). *Figure number changed and site numbers added.*

The benefits in harvest yield of agronomically-relevant grass-legume mixtures can be attributed to several reasons. First and most importantly, the mixture swards have access to symbiotically-fixed N_2 from the atmosphere through the legume component. Detailed analyses at one individual site of the pan-European COST 852 experiment (Nyfeler *et al.* 2011) demonstrated that symbiotic N_2 fixation of legumes is responsible for large quantities of N input into the system (see also chapter 6 of this deliverable), in full agreement with data from other, comparable systems (Boller and Nösberger 1987; Zanetti *et al.* 1997). Symbiotic N_2 fixation can only play an important role for overyielding if the legumes' growth and N_2 fixation perform well, and if nitrogen is a main limiting resource (Hebeisen *et al.* 1997). In particular N limitation is common in agronomic systems that target high forage yield and quality. For example, the maximum levels of 150 kg N ha^{-1} $year^{-1}$ applied at the productive sites of the pan-European experiment (Finn *et al.* 2013) have been shown to limit the performance of agronomically relevant grass species when grown in monoculture (Daepp *et al.* 2001; Nyfeler *et al.* 2009). Second, trait interactions other than between N_2 fixing legumes and non-fixing grasses have played a relevant role, because symbiotic N_2 fixation cannot explain the highly significant yield advantage of mixtures over legume monocultures (detailed results in Nyfeler et al. 2009). Rooting depth between some of the grasses and legumes in mixtures differs and enhanced nutrient uptake from a larger soil pool has been suggested to contribute to overyielding in mixtures (van Ruijven and Berendse 2003; de Kroon 2007; Roscher *et al.* 2008; de Kroon *et al.* 2012; Gastal *et al.* 2012). Third, agronomic species as used in the COST 852 experiment have been selected for high quantity and quality of yield. This contributes to a reduction in the magnitude of yield differences across monocultures of the different species, and reduces the expected negative effect (in comparison to the best-performing species) on mixture yield due to combining higher-yielding with lower-yielding mixtures. Fourth, mixtures as set-up in COST 852 strongly benefit from the combination of fast-establishing with more slow-growing, but temporally-persistent species (Kirwan *et al.* 2007; Finn *et al.* 2013); again, such a response is well recognised in other types of grassland (McKane *et al.* 1990). Fifth, characteristic within-season growth patterns that favour the grasses in spring during reproductive growth (Daepp *et al.* 2001) and the legumes in summer when temperatures are high (Vinther and Jensen 2000; Lüscher *et al.* 2005) could lead to temporal niches within the growing season that contribute to overyielding. In summary, these findings and underlying causes suggest that grass-legume mixtures offer great potential for increased production even at relatively low levels of species richness.

4. Positive legume effects operate over a wide range of legume proportions in mixtures

Studies that quantify the effects of species relative abundance on harvest yield are scarce, as most experiments assessing diversity effects on biomass production were set up with equal species proportions (e.g. “BioDepth” Hector *et al.* 1999; the ”Jena experiment” Roscher *et al.* 2004). Again, the pan-European grassland experiment of COST 852 allows an assessment of the effects of legume proportion on mixture yield, as mixture stands were established in greatly varying species proportions. It appeared that increasing evenness (the degree of equal species proportion) had a significant positive effect on harvest biomass yield (Kirwan *et al.* 2007; Finn *et al.* 2013), indicating that mixtures with 50:50% of grasses and legumes were, on average, amongst the best performing. More importantly, the positive mixture effect was persistent over a large range of legume proportion, as e.g. the yield of communities dominated by one species (70% sown proportion of one species) was comparable to that of the most even community (Finn *et al.* 2013), and this feature persisted over three years of experimental duration. Detailed analyses at the Swiss site demonstrated that the maximum yield of the mixtures was reached with a clover percentage in the sward between 36 and 70%, depending on the year and the N fertilizer treatment. Averaged over three years, clover proportions in mixtures ranging between 30 and 80% produced mixture yields higher than the best performing monoculture (Fig. 4, stands fertilised with 150 kg of N ha^{-1} year^{-1}) (Nyfeler *et al.* 2009). Thus, it did not matter whether the grass-clover mixtures were compared with the clover or grass monocultures: they were better than any of the monocultures. This result has high relevance as it highlights both the temporal stability of the mixture effect and its robustness over species proportions. This stability of high mixture yield in the face of compositional change is a most important feature for land managers and farmers because the benefit of grass-legume mixtures can be achieved despite a considerable change in relative proportions of the involved species (Connolly *et al.* 2013; Finn *et al.* 2013).

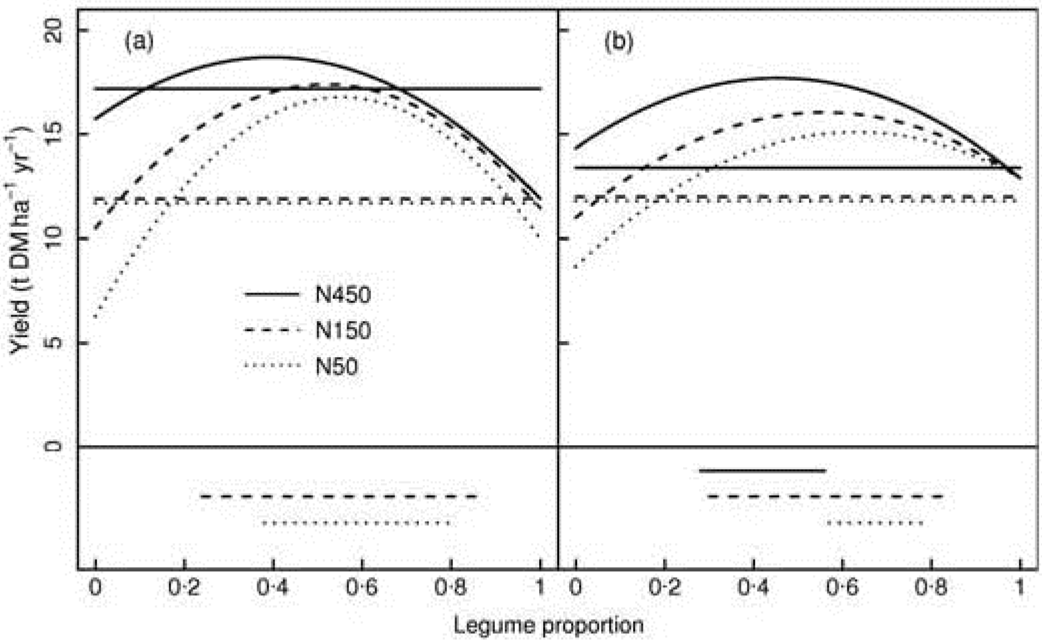

Fig. 4. Predicted mixture yield for increasing legume proportions and the three N fertilisation levels in the second year (a) and over all 3 years (b). Predictions are based on regression analysis, and are displayed for mixtures with equal proportions of the two legume species and equal proportions of the two grass species. Highest monoculture yield is indicated by horizontal lines[1] (above the x-axis). Horizontal lines below the x-axis indicate the range of significant transgressive overyielding ($P \leq 0{\cdot}05$), that is, mixture yield being significantly higher than the highest monoculture yield.

[1] In the second year, *T. repens* at N50 and N150 and *D. glomerata* at N450; over all 3 years *T. pratense* at N50 and N150 and *D. glomerata* at N450

This figure is provided (with permission) from Nyfeler *et al.* (2009). *Figure number changed and legend shortened.*

5. Options for maintaining legume abundance in swards

Legumes have a distinct competitive advantage in N-limited systems (Hartwig 1998). Grasses supply their demand for N solely by uptake of mineral N in the soil; if soil N levels are low, then the growth rate of grasses will be slow. In contrast, legumes avoid N deprivation by supplementing mineral-N uptake with symbiotic N_2 fixation, thereby retaining a relatively high growth rate even in an environment with low levels of soil N (Woledge 1988; Schwinning and Parsons 1996a). However, where mineral N is abundant, N_2 fixation is energetically costly and N_2 fixers tend to be outcompeted by non-fixing species (Soussana and Tallec 2010).

The process of N_2 fixation carried out by legumes adds variable amounts of N to the soil, depending mainly on the amount of legume present but also on environmental constraints (Wu and MeGechan 1999). Variability in the processes of N_2 fixation by legumes, transfer via microbial processes, and uptake by associated grasses result in a degree of uncertainty in predicting the soil N status in mixed swards. Schwinning and Parsons (1996a, 1996b) described an intrinsic oscillatory component of variation in grass-legume systems, which, together with external stimulants of legume yield variation, interacts to produce a random element to species balance. The key to understanding the source of yield variability in grass-legume mixtures is the difference in these functional groups' response to N in the soil. The contrasting responses of grasses and legumes to the availability of soil N provides one explanation for their sustainable coexistence: in conditions of low soil N legumes have a growth advantage and should exclude grasses; in conditions of higher soil N grasses have a competitive advantage and should exclude legumes (Soussana and Tallec 2010). Thus, in conditions of stable soil N, Schwinning and Parsons (1996a) argued that these species could not coexist. However, soil N level is a dynamic variable that not only affects plants, but is also affected by them. In conditions of low soil N the dominance of legumes causes N to accumulate in the soil, which eventually promotes dominance of the grass component (the so-called 'paradox of enrichment'). Schwinning and Parsons (1996a) stated that this negative feedback control of soil N status maintains the competitive balance between grasses and legumes in mixed swards. However, the control of soil N is a relatively slow process, and models have shown that grasses and legumes can oscillate in dominance for many years before equilibrium is reached (Schwinning and Parsons 1996b). Moreover, much of the evidence concerning fluctuations in grass-legume abundance is derived from ryegrass-clover mixtures. Evidence from other systems is sparse, and mixtures such as grass-lucerne could be more unstable and less flexible.

For farmers, variability in legume abundance translates into uncertainties in herbage production and forage quality, so for this reason it is vital to understand the complexities of grass-legume interactions. Control of the synchrony of N supply and demand in grass-legume swards appears to be the key to achieving sustainable grassland agriculture. The issue may be approached through improving plant uptake of N from the soil, and by manipulating soil N supply. The former can be approached through manipulation of the species content of grassland mixtures via the use of perennial forage species with different spatial and temporal nutrient uptake patterns (Daepp *et al.* 2001; Lüscher *et al.* 2005). Achieving the latter objective is more problematic, due to large gaps in current knowledge of plant-driven N fluxes (Høgh-Jensen 2006). For example, legume species differ substantially in the amount of N they add to grassland systems through N_2 fixation, and in the amount of inorganic soil N they take up. For example, the widely-grown temperate forage legume derives a smaller proportion of N from atmospheric nitrogen than other forage legumes due to the high uptake of soil inorganic N by its deep and extensive root system (Blumenthal and Russelle 1996; Rasmussen *et al.* 2012). Furthermore, lucerne seems to transfer a lower proportion of fixed N to the rhizosphere and companion grass species than other legumes (Brophy and Heichel 1989; Lory *et al.* 1992).

Probably due to the challenging control of the synchrony of N supply and demand, legume proportions in mixed swards have been observed repeatedly to decrease over time (Frame 1986; Elgersma and Schlepers 1997; Guckert and Hay 2001), the decline in relative legume abundance being considerably accelerated under higher levels of N fertilisation (Hebeisen *et al.* 1997; Aydin and Uzun 2005; Nyfeler 2009). This matches well the remarkable decrease in legume proportion of mixture swards throughout the three years of experimental duration in the pan-European COST 852 experiment (Finn *et al.* 2013). The big challenge for legume-based grassland-livestock systems is therefore to maintain the proportion of legumes within an optimal range. Four of the most promising strategies are outlined below.

5.1 Fertiliser application

Sward management strategies that reduce N fertilizer input can stabilise the proportion of white clover in permanent grassland (Schwank *et al.* 1986; Ledgard *et al.* 2001). The effectiveness of reduced N fertilisation in regulating the proportion of white clover is, for example, evident from the Swiss Free-Air CO_2 Enrichment (FACE) experiment (Hebeisen *et al.* 1997; Zanetti *et al.* 1997): averaged over the first three years, the contribution of white clover was 57% at low levels of N fertilisation (100 and 140 kg N ha^{-1} $year^{-1}$, combined with frequent defoliation), whereas it was only 14% with high N fertilisation (420 and 560 kg N ha^{-1} $year^{-1}$, combined with infrequent defoliation). Further evidence comes from the Swiss site of the earlier mentioned COST 852 project, where three different levels of mineral-N (50, 150, 450 kg N ha^{-1} $year^{-1}$) were applied to the grass-clover mixtures (two grass and two clover species) managed for three consecutive years. Here, N fertilisation strongly affected clover proportion: averaged over all mixtures receiving 50 kg N ha^{-1} $year^{-1}$, the two clover species (sum of both) achieved 42, 56, and 24% proportional biomass in years 1, 2, 3, respectively; however, fertilized with 450 kg N ha^{-1} $year^{-1}$, clover proportions were only 32, 24, and 5%. Similar results were found by Nassiri and Elgersma (2002), where in only one growing season the application of 150 kg N ha^{-1} $year^{-1}$ reduced the clover content in dry matter harvest of ryegrass-white clover mixtures to 12% compared to 43% without N application. In a similar system with respect to species and N application, the average difference in the mixture clover proportion between the N treatments was 25% (59% *vs.* 34% with zero N and 165 kg N ha^{-1} $year^{-1}$, respectively; Elgersma *et al.* 2000). Most recently, Oberson *et al.* (2013), assessed the dynamics of N_2 fixation and N transfer in grass-clover leys under different cropping systems (organic, conventional). Irrespective of the cropping system, clover proportion was greater under reduced N fertilisation and reached 47% in the unfertilised control, while it decreased to 30% in a reference treatment that received 120 kg of N ha^{-1} $year^{-1}$ (averaged over two years). It is most relevant to note that high productivity levels can still be maintained at moderate to intermediate levels of N fertilisation. For example, Nyfeler *et al.* (2009) found yields of grass-clover mixtures fertilized with 50 or 150 kg of N ha^{-1} $year^{-1}$ to be as high (Fig. 4a) or even higher (Fig. 4b) than the yields of the heavily fertilized (450 kg of N ha^{-1} $year^{-1}$) monocultures of the grasses *Lolium perenne* and *Dactylis glomerata*.

Regarding nutrients other than N, in particular phosphorus (P), potassium (K), and sulphur (S), their availability is crucial for growth and N_2 fixation of legume species (Brown *et al.* 2000; Tallec *et al.* 2009; Batterman *et al.* 2013). For example, both N_2 fixation and plant growth have been shown to be constrained by P in native, phosphorus-poor soils of the tropics (Batterman *et al.* 2013) and in nutrient solution (Almeida *et al.* 2000). In a similar way, limitations in the availability of soil S significantly restricted the growth of clover in experimental ryegrass-white clover mixtures receiving 180 kg N ha^{-1} $year^{-1}$ (Tallec *et al.* 2009).

In conclusion, the available evidence suggests that, in temperate grassland systems, legume abundance in mixtures can be balanced through adjusting N fertilizer application. It is most promising that even substantial reduction in N fertilizer application is not expected to impact negatively on productivity, because a potential N deficiency due to reduced fertilisation is counteracted by increased symbiotic N_2 fixation of legumes in mixture swards (see chapter 6).

5.2 Defoliation frequency

In well-managed grassland systems, defoliation frequency is closely related to fertilisation rates. Nevertheless, adapting the frequency of defoliation provides an additional management strategy to adjust the legume proportion in swards. Most evidence to this option comes from white clover, the most promising legume for mixture swards in temperate, northern and upland Europe (Guckert and Hay 2001). White clover is resistant to frequent defoliation because it has stolons that grow closely at the ground surface and escape the harvest of petioles and leaves. Therefore, more frequent cutting will reduce competition for light from companion grass species and thereby promote growth of clover. The earlier-cited FACE experiment manipulated not only CO_2 partial pressure and N fertilisation, but also defoliation frequency. It became evident that, throughout all treatments and years, increased defoliation raised the clover proportion in mixed swards (Hebeisen *et al.* 1997). Further studies confirm the relationship between cutting and relative abundance of white clover (Schwank *et al.* 1986; Elgersma and Schlepers 1997). For example, Elgersma and Schlepers (1997) examined grass-clover mixtures that were cut in two frequencies over three years: the more frequently harvested sward had finally clover proportions of 50%, while it was only 42% with less frequent cutting. Similarly, Wen and Jiang (2005) found a tendency for higher proportions of white clover in mixed swards with increased cutting frequency. Their experiment ran for two years and tested a ryegrass-white clover pasture under four different cutting frequencies (8 cuts year^{-1}, 4, 2, 1): the final proportion of white clover was *ca.* 20% in the most frequently cut plots, while it was less than 10% with one cut per year. Regarding pasture legumes other than white clover, only few information is available to effects of defoliation frequency on mixture legume proportion (but see Peterson *et al.* 1994; Giambalvo *et al.* 2011).

Although the influence of defoliation frequency on clover proportion in mixed swards appears to be smaller than the effects of N application (Schwank *et al.* 1986; Hebeisen *et al.* 1997), adjustment of cutting and grazing rates is one of several options to keep clover proportions in swards at stable levels.

5.3 More complex mixtures

5.3.1 Number of species

A further option to stabilise legume abundance in mixtures is an optimised species composition of seed mixtures that prevents competitive dominance. Improved seed mixtures will require decisions on how many and which species (or variety) to include, and which proportions of the species to choose. The potential of such improved mixtures is, for example, evident from the Swiss site of the COST 852 project, where, besides the experimental four-species mixtures (Kirwan *et al.* 2007; Nyfeler *et al.* 2009), Swiss Standard Mixtures (SSMIX, Suter *et al.* 2012) were also examined. These mixtures contain more species (up to eight) and their composition (relative and absolute abundances of species) has been continuously improved over several decades to maintain stable species composition based on experiments and observations on farms. Over the three years of experimental duration, the decline in clover abundance in the SSMIX was much smaller than that in the four-species mixtures of the pan-European experiment (Fig. 5a) and, at the same time, SSMIX were more productive throughout (Fig. 5b).

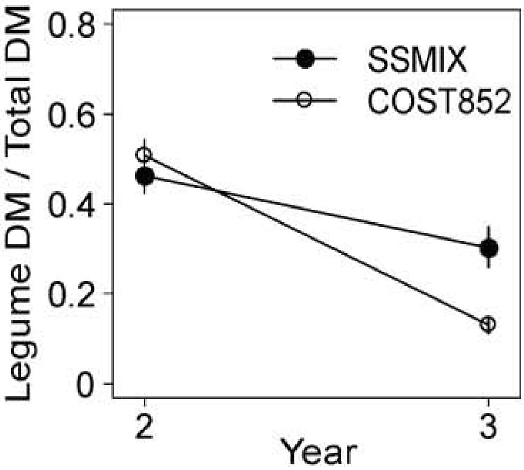

Fig. 5a. Legume proportion of total spring-DM (dry matter) yield of COST852 and SSMIX mixtures (bars = 1 SE).

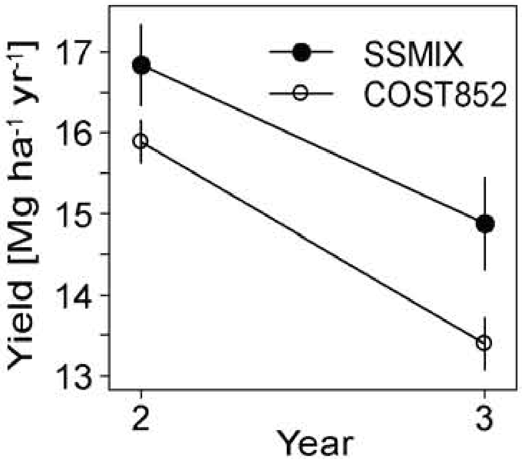

Fig. 5b. Annual yield of COST852 and SSMIX mixtures for the second and third experimental year (bars = 1 SE).

Figures are provided (with permission) from Suter *et al.* (2010). *Figure numbers changed.*

Current concepts suggest that combining species with high “combining ability” in more complex mixtures would result in better resource exploitation through niche differentiation (Aarssen 1983; Hill 1990), and empirical evidence supports this idea (Hooper *et al.* 2005; Roscher *et al.* 2005; Nyfeler *et al.* 2009; Finn *et al.* 2013). In comparison, combining species or genotypes in mixtures that have comparable “competitive abilities” would result in more balanced and stable mixtures (Evans *et al.* 1985, 1989; Turkington 1989a; Lüscher *et al.* 1992). Both existing theory and experimental evidence on combining species in more complex mixtures is intriguing and call for extended research on how such mixtures can stabilise legume proportion and increase total yield in agricultural grassland systems.

5.3.2 Genetic diversity within species

Not only do species differ in their competitive abilities, so also do cultivars within species. For example, 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. In that context, the presence of a large degree of genetic diversity for morpho-physiological traits in natural plant populations might suggest that the use of blends of several cultivars and thus of composite populations with a high degree of heterogeneity would also be advantageous under agricultural conditions. However, there is relatively limited evidence to support this idea. It has been demonstrated that greater heterogeneity in genotype morphology in white clover populations failed to translate into higher dry matter production (Annicchiarico and Piano 1997), comparable to results obtained with cultivar mixtures on perennial ryegrass (Surault *et al.* 2010). A similar result was obtained by Williams *et al.* (2003), also for white clover, although there was some evidence that yield stability over the duration of the experiment was increased in the mixture containing a blend of cultivars. The impact of increased heterogeneity in legume populations on their performance in grass-legume mixtures was also investigated by Collins *et al*. (2004). It was hypothesised that ‘wide genetic base’ (WGB) composite populations of red and white clover, comprising mixtures of seed of many varieties, would provide a platform for sustained positive effects on harvest yield in grass-legume mixtures; however, no yield advantage was observed in mixtures with WGB populations (Collins *et al.* 2004). A detailed study of the influence of the WGB legumes on sward dry matter productivity and unsown species invasion in complex mixtures was carried out at the COST 852 site in southern Sweden (Frankow-Lindberg *et al.* 2009). It was found that the impact of the WGB populations on both these ecosystem properties was small compared with the diversity effect brought about by the mixing of species. This could be due to the fact that traits are more likely to be similar within species (even in populations with deliberately enhanced heterogeneity) than between different species. Another explanation for the weak effect of the WGB composites might be a reduction in intra-specific genetic diversity caused by natural selection over time of the most adapted individuals for the site in challenging environments such as the Swedish COST site. The phenomenon of genetic shift was observed in a genome-wide analysis of population genetic differentiation in the WGB and single-cultivar germplasm in Northern European COST 852 sites (Collins *et al.* 2012). Thus, the use in diverse species mixtures of WGB populations containing site-adapted germplasm may produce more positive results in terms of sward yield.

In summary, these results demonstrate that the species composition of multi-species seed mixtures offers a multi-factorial opportunity for optimization, but that the use of blends of cultivars might provide only limited benefits. Further research is needed to clarify the role of blends of cultivars in mixed grass-legume systems before such on option can generally be recommended.

5.4 Selection of different legume species and breeding of adapted cultivars

Under climatic conditions of low winter temperatures, limited water availability or severe drought, the long-term growth of red and white clover appears to be restricted (Sturludóttir *et al.* 2013; unpublished results). Here, selecting different legume species adapted to harsh environmental conditions and/or specific breeding programs for suitable cultivars can significantly contribute to higher persistence of legumes in grassland, and thus to more stable legume abundance in swards (Serraj *et al.* 1999; Patto *et al.* 2006; Carbonero *et al.* 2011). For example, evaluation of two white clover cultivars at twelve sites over a European gradient revealed significant differences in clover mixture proportion following overwintering, much of the difference being due to the cultivars’ varying response to temperature (daily means) (Wachendorf *et al.* 2001). Frankow-Lindberg (2001) tested nine genetically different populations of white clover for their physiological response to frequent sub-lethal frost in northern climates. She found not only relevant differences among populations, but concluded that there would be sufficient genetic variation in at least one population for further trait selection in the field. The research of Collins *et al*. (2012) on genetic change in legume germplasm (commercial cultivars and experimental populations) further supported this hypothesis; the authors found that measurable genetic shift occurred in both red and white clover over a relatively short time period when grown in cold sites in Northern Europe. The degree of genetic change that occurred depended on how well adapted the populations initially were to their growing environment, as well as on the climatic characteristics of the site itself. One practical implication of these results is that it would probably be beneficial for legume breeding programmes aimed at producing cultivars for challenging environments to consider collecting germplasm from survivor populations in these areas in order to reinforce existing breeding lines.

Newly selected legume species and cultivars should be evaluated in combined growth with grasses, either in single species combination or in more complex mixtures as outlined above. Current European initiatives Legume Plus (www.legumeplus.eu) and MultiSward (www.multisward.eu), all within the seventh frame framework program, deal with such requirements.

6. Legumes are responsible for large N inputs into the soil-vegetation system by symbiotic N_2 fixation

The major benefit of legumes to more sustainable but still productive grassland systems comes through their ability to incorporate large amounts of N into the system. In temperate and Northern grassland, amounts of symbiotically fixed N_2 (N_{sym}) by legumes can range from 100 to 380 kg of N ha^{-1} $year^{-1}$, but values as high as 500 kg of N_{sym} ha^{-1} $year^{-1}$ have also been reported (Boller and Nösberger 1987; Ledgard and Steele 1992; Carlsson and Huss-Danell 2003; Lüscher *et al.* 2013; Oberson *et al.* 2013). Amounts of N_{sym} by *crop* legumes are smaller and range between 30 and 150 kg of N ha^{-1} $year^{-1}$, with maximal values around 200 kg of N_{sym} ha^{-1} $year^{-1}$ (Peoples *et al.* 2009). In agricultural grass-legume mixtures, legumes can acquire more than 80% of their own N demand through symbiosis (Boller and Nösberger 1987; Zanetti *et al.* 1997; Nyfeler *et al.* 2011; Rasmussen *et al.* 2012; Oberson *et al.* 2013), and consequently, the relative availability of soil N increases for grasses due to 'N-sparing'. Moreover, in mixed grass-legume systems, amounts of 10-75 kg of N ha^{-1} $year^{-1}$ are transferred from legumes to grasses (Høgh-Jensen and Schjoerring 1997; Zanetti *et al.* 1997; Nyfeler *et al.* 2011), while the amounts of transferred N depend on the donor and the receiver plant species (Pirhofer-Walzl *et al.* 2012). In a recent analysis of the amount of symbiotic N_2 fixation within the pan-European COST 852 project (Suter *et al.* 2013; unpublished results), the average amount of N_{sym} in 50:50% grass-legume mixtures across all evaluated sites was 106 kg ha^{-1} $year^{-1}$; however, maximal N_{sym} in mixtures at individual sites and years was as high as 325 kg ha^{-1} $year^{-1}$. Further, across sites, mixtures with one third of legume proportion attained amounts of N_{sym} that were not significantly different from maximal amounts in mixtures with much higher legume proportion (including legume pure stands), suggesting that grassland with largely varying legume proportions can achieve substantial gain from the process of symbiotic N_2 fixation (Fig. 6).

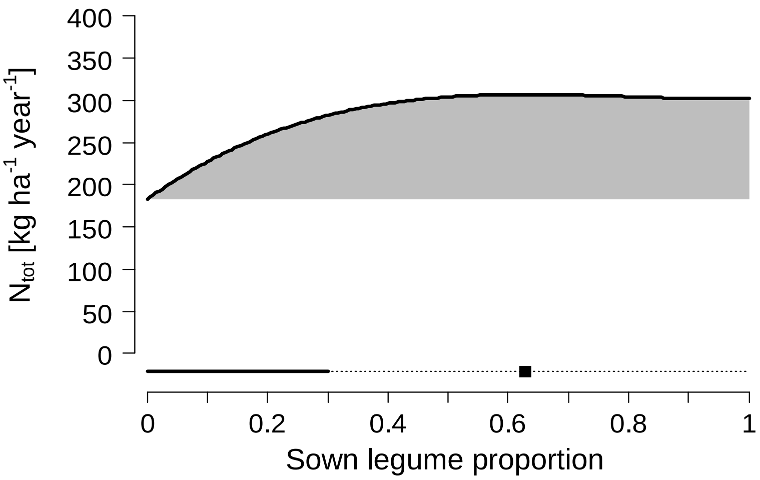

Fig. 6. Total nitrogen yield (N_{tot}) as affected by sown legume proportion at the first year of a multisite grassland experiment across Europe. The curved bold line displays the predicted N_{tot} across 15 sites, which spanned a gradient of climate from Atlantic to continental, and from temperate to arctic. The grey shaded area indicates N derived from symbiotic N_2 fixation of legumes across all sites based on the N difference method (Ledgard and Steele 1992). The horizontal bold line at bottom indicates the range of legume proportion for which N_{tot} was significantly smaller ($P \leq 0.05$) than at maximum (■); consequently, the dotted line displays the non-significant range.

In mixtures containing ryegrass, cocksfoot, red and white clover and being fertilised with 150 kg N ha^{-1} year^{-1}, Nyfeler *et al.* (2011) found maximum N_{sym} values of 295 kg N ha^{-1} year^{-1} (average over two years, Fig. 7). More importantly, total harvested dry matter yield (Nyfeler *et al.* 2009) and total yield of harvested N (Nyfeler *et al.* 2011) were maximal at intermediate levels of clover proportion of 40-60% in mixture. Interestingly, Nyfeler *et al.* (2011) found stimulatory effects of the accompanying grasses on the symbiotic N_2 fixation activity of clover. 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) a clover proportion of 40-60% in the mixture was sufficient to attain the same amount of N from symbiosis as in pure clover stands. 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 limited because 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_2 fixation was down-regulated. This was due to the clovers' adequate access to mineral N sources because of low grass abundance in addition 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).

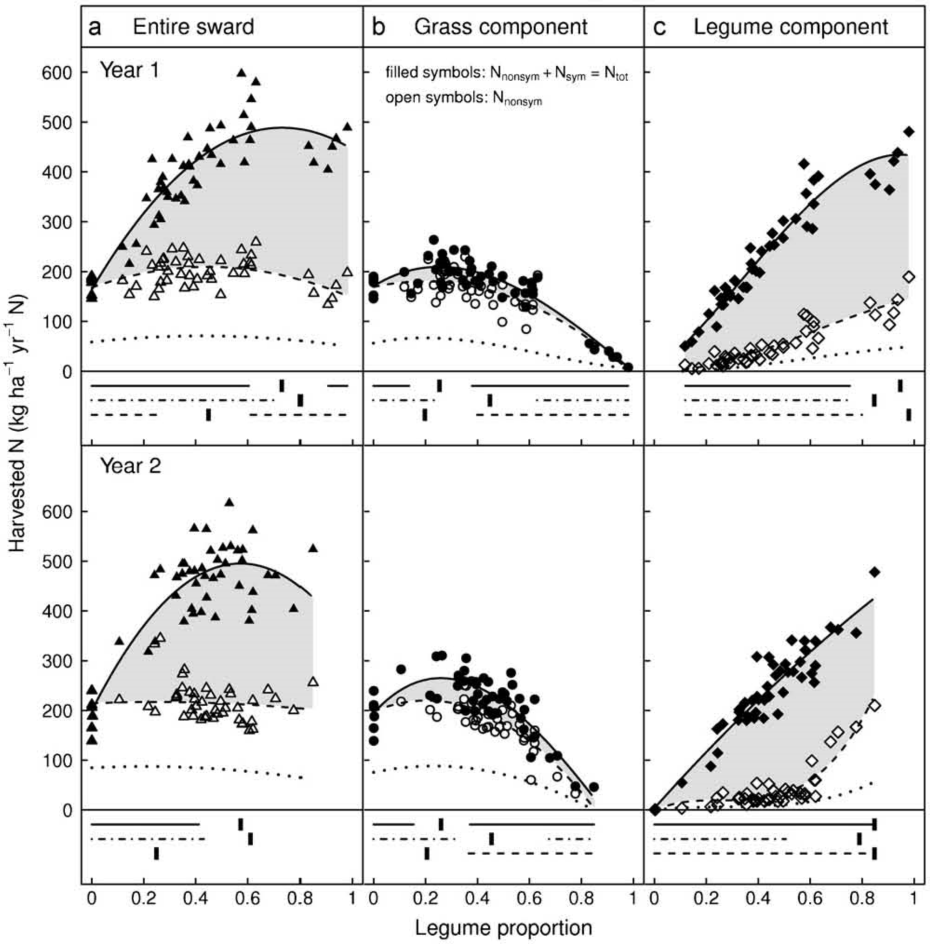

Fig. 7. Nitrogen yield and its fractions harvested in the entire sward (a) or in either its grass (b) or legume (c) component as affected by the legume proportion in moderately fertilised swards (150 kg N ha^{-1} year^{-1}) for the first and second experimental year. Curves indicate N from fertilizer (N_{fert}, · · ·) and N from non-symbiotic sources (N_{nonsym}, - - -). N_{nonsym} is the sum of N_{fert} plus N derived from soil organic matter (N_{soil}). The shaded area is the difference of N_{nonsym} to total harvested N (N_{tot}, —) and represents N from symbiotic sources (N_{sym}), which comprises of N from symbiotic N_2 fixation in the legume component (N_{symfix}), N_{symfix} transferred to the grass component ($N_{symtrans}$), and the sum of both in the entire sward. Horizontal lines below the curves indicate the range of legume proportions for which N_{nonsym} (- - -), N_{sym} (·–·–·) and total harvested N_{tot} (—) are significantly smaller ($P \leq 0.05$) than at maximum (▌).

This figure is provided (with permission) from Nyfeler *et al.* (2011). *Figure number changed and legend shortened.*

It has been suggested that the degree of symbiotic N_2 fixation of legumes is controlled by a series of eco-physiological triggers and N feedback mechanisms from the individual plant to the ecosystem level (Schwinning and Parsons 1996b; Hartwig 1998; Soussana and Tallec 2010), with N_2 fixation of legumes being largely regulated by the level of site productivity, i.e. the N sink strength (N-demand) of the whole system. Following this concept, legumes would account for the gap between N-demand (sink) and N-availability (source) from non-symbiotic N-sources by adjusting the degree of symbiotic N_2 fixation (Hartwig 1998; Soussana and Tallec 2010; Lüscher *et al.* 2011). Based on experimental results, mechanistic modeling could tightly couple symbiotic N_2 fixation in legume-based grassland systems to the N demand at scales ranging from the individual plant to the grassland ecosystem (Soussana and Tallec 2010). For example, it was modeled that N uptake rates of legumes and grasses under varying soil N availability at the patch scale could lead to stable grass-legume co-existence at the community level (Soussana and Lafarge 1998).

The growth and ability of legumes to fix nitrogen may be hampered in climatic conditions with low winter temperatures and/or scarce precipitation (Nesheim and Boller 1991; Serraj *et al.* 1999). It is known from controlled experiments that low temperatures and severe water stress negatively affect the process of nitrogen fixation-nodulation (e.g. Roughley and Dart 1970) and nitrogenase activity (e.g. Cralle and Heichel 1982; Durand *et al.* 1987). However, only a few field studies have been published on symbiotic N_2 fixation under marginal conditions such as high altitude (e.g. Bowman *et al.* 1996; Jacot *et al.* 2000a) or high latitude (e.g. Henry and Svoboda 1986; Sparrow *et al.* 1995). Jacot *et al.* (2000a, 2000b) studied symbiotic N_2 fixation of naturally occurring legumes and the N balance of the whole grassland ecosystem in species-rich semi-natural pastures in the Swiss Alps along a gradient from 900 m a.s.l. to the altitudinal limit of legume occurrence at 2600 m a.s.l. Investigated legumes were: *Lotus alpinus* (DC.) Schleicher, *Lotus corniculatus* L., *Trifolium alpinum* L., *Trifolium badium* Schreber, *Trifolium nivale* Sieber, *Trifolium pratense* L., *Trifolium repens* L., *Trifolium thalii* Vill., *Vicia sativa* L. Absolute amounts of N_{sym} decreased significantly with increasing altitude (from 18 to 1 kg N ha^{-1} $year^{-1}$) in conjunction with a reduction of the total productivity of the community and with a decrease in sward legume proportion from 15 to 4%. These features went along with a decline in summer precipitation, mean temperatures, and a decrease in soil pH. In the context of regulation of N_2 fixation, results of Jacot *et al.* (2000a) are in agreement with Hartwig (1998) and Soussana and Tallec (2010) that predict lower symbiotic N_2 fixation at low productivity levels. However, although absolute levels of N_{sym} were closely related to productivity and pedo-climatic factors, the percentage of N_{sym} in legumes was not (Jacot *et al.* 2000a): % N_{sym} in legume plants (59 to 90%) was not diminished over the altitudinal range, most probably due to the high abundance of non-legumes (grasses, forbs) that competed strongly for mineral N in soil. Results of Jacot *et al.* (2000a) indicate a substantial contribution of symbiotic N_2 fixation to the legumes' N requirements over varying environmental conditions, suggesting that - within restrictions of low temperatures (Cralle and Heichel 1982) and severe water stress (Durand *et al.* 1987; Serraj *et al.* 1999) - the physiological process of symbiotic N_2 fixation in naturally-occurring species is adapted to pedo-climatic conditions. Interestingly, results of N_2 symbiosis in the COST 852 project agree with such conclusion (Suter *et al.* 2013; unpublished results). Here, the proportion of N_{sym} to the total N yield of sites was, on average, not correlated with site productivity (within the conditions of nitrogen application across sites, ranging from 0 to 150 kg N ha^{-1} $year^{-1}$), but negatively affected by low annual precipitation and minimal site temperature. This provides evidence from a European gradient of sites that agricultural grassland systems of largely differing productivity levels can benefit from N_2 fixation, given that legumes can be grown in mixtures and that symbiotic N_2 fixation remains active in legume plants.

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.

1. Grass-legume mixtures can potentially fix more N_2 from the atmosphere than legume monocultures.

There is no trade-off between high productivity and high gains of nitrogen from symbiotic N_2 fixation 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 (Sangakkara *et al.* 1996; Hartwig 1998; Brown *et al.* 2000; Tallec *et al.* 2009; Batterman *et al.* 2013).

2. Even though high inputs of N into the ecosystem from symbiosis can cause a risk for N losses into the environment (Loiseau *et al.* 2001; Scherer-Lorenzen *et al.* 2003), such 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 (no less than 40% of sward biomass occupied by grasses) (Nyfeler 2009; Nyfeler *et al.* 2011; Lüscher *et al.* 2013) (see also Chapter 7).

7. Legume-based forage systems can reduce negative impacts of livestock production on the environment

The great opportunities of legumes for environmentally friendly yet productive grassland-livestock systems derive from different features: (i) increased yield, (ii) replacement of mineral-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. The 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 unit of the final product. This makes legumes to a key option for sustainable intensification of agriculture.

7.1 Forage legumes can reduce nitrate leaching

In comparison to fertilized pure ryegrass stands (300 kg N ha^{-1} $year^{-1}$), the presence of legumes in mixed swards can reduce or stabilise nitrate (NO_3) leaching to groundwater while, at the same time, reduce the need for mineral N fertilizers (Eriksen *et al.* 2004). For example, Vertès *et al.* (1997) found a 5 to 10% reduction of NO_3 leaching under grass-clover receiving no fertilizer compared to fertilized (250 kg N ha^{-1} $year^{-1}$) pure ryegrass pastures; both systems had comparable biomass yields (8.7 t ha^{-1} $year^{-1}$ for ryegrass-white clover, 9.5 t ha^{-1} $year^{-1}$ for pure grass). At similar stocking rate (3.3 cows ha^{-1}) and milk yield per hectare, Ledgard *et al.* (2009) reported identical levels of N leaching (30 kg N ha^{-1} $year^{-1}$) for grass-clover pastures receiving no mineral N fertilizer as for pure ryegrass pastures fertilised with 160 kg N ha^{-1} $year^{-1}$. However, losses of nitrate under grazed grass-clover swards can increase with increased clover proportion (Schils 1994), supplemental N fertilisation (Ledgard *et al.* 1999; Ledgard *et al.* 2009) or high stocking rates (Simon *et al.* 1997; Eriksen *et al.* 2010). Regarding legumes other than clover, NO_3 concentrations in ground water were found to be lower under lucerne than under annual crops (Russelle *et al.* 2001; Russelle *et al.* 2007) and grass-clover mixtures (Ledgard *et al.* 2009).

However, very high inputs of N from symbiosis into grassland systems can increase the risk of nitrate leaching (Hooper and Vitousek 1998; Loiseau *et al.* 2001; Scherer-Lorenzen *et al.* 2003). 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^{-1}) than with pure perennial ryegrass (1 to 10 kg N ha^{-1}); yet, much higher values were reported for bare soils (84 to 149 kg N ha^{-1}). Nevertheless, it can be expected that N losses by leaching will be prevented as long as the percentage of grass in mixed grass-legume swards is well balanced to take up mineral N from the soil (no less than 40% of sward biomass occupied by grasses). For example, under a mowing regime and 150 kg N fertilizer input ha^{-1} $year^{-1}$, Nyfeler (2009) found an increased risk of nitrate leaching only if the percentage of legumes in the mixture exceeded 60-80%. Only few studies have assessed changes in soil nitrate content under mixtures containing legumes for periods of more than a few years. In the long-term, levels of soil nitrate might rise due to the mineralization of nitrogen-rich legume residues. During a five-year measurement period in an unfertilized experimental grassland, the mere presence of legumes resulted in increased soil NO_3-N content but the legume effect on nitrate in leaching water did not change over time, suggesting no increased risk of N leaching (Oelmann *et al.* 2011).

7.2 Forage legumes can contribute to reduced greenhouse gas emissions

7.2.1 Methane

Methane produced in the rumen is a large contributor to greenhouse gas (GHG) emissions by livestock systems (Tamminga *et al.* 2007; Rowlinson *et al.* 2008; Waghorn and Hegarty 2011). Legumes can contribute to reduced ruminal methane production per unit of intake. Ruminants fed legume forages generally emitted less methane than grass-fed animals, per unit of feed intake (McCaughey *et al.* 1999), although not in all cases (van Dorland *et al.* 2007; Hammond *et al.* 2013). Inconsistency of results between experiments can arise from difference in forage composition (forage species, stage of maturity, presence of condensed tannins) and animal genotypes. Condensed tannins (CT) may also be useful for reducing greenhouse gases (Kingston-Smith *et al.* 2010), as several studies have shown that CT reduced methane production *in vitro*. A recent meta-analysis revealed a general anti-methanogenic effect of CT above 20 g kg^{-1} dry matter in feeds (Jayanegara *et al.* 2012). Some effects of CT to lessening methane emissions were also reported from *in vivo* studies with *Onobrychis viciifolia* Scop. (sainfoin, Waghorn 2008), *Lotus corniculatus* L. (birdsfoot trefoil, Woodward *et al.* 2004) and *Hedysarum coronarium* L. (sulla, Woodward *et al.* 2002). Both sainfoin and sulla have higher CT contents than the widely used red and white clover and lucerne, which are low in CT (Häring *et al.* 2007; Häring *et al.* 2008). Therefore, CT related effects on methane emissions can only be achieved by cropping the above mentioned CT containing legume species, which, up to now, are only rarely cropped in Europe.

7.2.2 Nitrous oxide

Each kg of N as ammonium nitrate produced in the industrial Haber-Bosch process emits significant amounts of greenhouse gases (ecoinvent Centre 2010), of which 19 g consists of N_2O, a greenhouse gas that is *ca.* 300 times more active than CO_2 (Kingston-Smith *et al.* 2010). In addition, the IPCC (2006) suggested that for each of 100 kg of N fertilizer added to the soil, on average 1.0 kg of N is emitted as N_2O. The process of denitrification is the most important source of N_2O from pasture systems (Soussana *et al.* 2010) and large peaks of N_2O emissions are measured in grassland immediately after N-fertilizer applications (Ineson *et al.* 1998; Klumpp *et al.* 2011). There are four reasons why N_2O emissions (as other N losses like NO_3 leaching) 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_2 fixation activity is down-regulated if the sink of N for plant growth is small, (iii) in balanced grass-legume mixtures the grass roots take up N derived from legume roots and from mineralization of soil organic matter, and (iv) pure grass swards are generally fertilized with inorganic N, and application of N fertiliser in itself leads to N_2O losses. A recent review 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 (8 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 because of a lack of evidence of significant emissions arising from the fixation process itself (Rochette and Janzen 2005). Emissions of N_2O from legumes can 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) used life-cycle analysis to show that greenhouse gas emissions decreased by 1.15 to 1.00 kg eq-CO_2 kg^{-1} milk with mixed grass-clover pastures compared to pure grass pastures. 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 essentially relies on permanent grass-white clover pastures, which are grazed all year round with an annual N fertilizer input of 100 kg ha^{-1} and less than 10% of the feed requirement of cows is provided by feed supplements. The authors showed that the total emission per kg milk is 30 to 80% lower for the New Zealand system than for intensive European dairy farms based on predominantly grass pastures. Schils *et al.* (2005) compared the total emissions from dairy systems in the Netherlands, which were either fertilized ryegrass or grass-clover pastures (inorganic-N inputs of 275 and 69 kg ha^{-1} $year^{-1}$, respectively): greenhouse gas emissions per kg of milk were 10% lower for grass-clover pasture-based systems than for the ryegrass monocultures.

7.2.3 Carbon dioxide

Industrial production of each kg of inorganic N emits 2.25 kg of CO_2 (ecoinvent Centre 2010). In this respect, legumes offer a big advantage because the entire carbon (C) needed for symbiotic N_2 fixation comes directly from the atmosphere via photosynthesis and this process is ‘greenhouse-gas neutral‘.

A further aspect mitigating climate change is C sequestration into the soil (Soussana *et al.* 2010). Carbon can only be introduced into the soil via plant photosynthesis 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 indicates 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). Legumes and their symbiotic N_2 fixation thus offer the option to couple C and N cycles and deliver the N needed to sequester C into soil organic matter (Zanetti *et al.* 1997; Lüscher *et al.* 2004). 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 (300-500 kg C ha^{-1} $year^{-1}$ over a time span of 20 years). Indeed, several studies found higher soil organic matter contents under mixed grass-legume swards than under pure grass swards (Mortenson *et al.* 2004; Fornara and Tilman 2008).

7.3 Forage legumes reduce consumption of non-renewable energy

The introduction of legumes reduces non-renewable energy consumption in livestock farming since legume-based grassland systems access atmospheric N and no direct financial or energetic cost is linked to such 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). Further, it is 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^{-1}, but only 0.06 MJ with ryegrass-white clover mixtures, 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 higher amounts of N fertilizer on pure ryegrass pastures, in comparison with the lower use of N in New Zealand systems.

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Annex 1: Supporting information

Table A1. Information on sites, and basic management and weather information. Sites are distinguished by their identifier number in the database from which these data are derived, and are arranged in order of declining total yield of the best-performing monoculture over the three years (as in Fig. 2).

This table is provided (with permission) from Finn *et al.* (2013). *Table number changed and header shortened.*

Site # in database	Country	Site	Latitude	Longitude	Altitude (m a.s.l.)	Nitrogen fertilizer (kg ha^{-1} per annum)	Harvests per annum (year 1,2,3)	Annual rainfall (mm)	Annual mean temp (^{o}C)	No. of years
1	Belgium	Merelbeke	50^{o}59'N	3^{o}49E	11	150	4,3,4	780	9.9	3
40	Slovenia	Ljubljana	46^{o}03'N	14^{o}28'E	300	120	4	1147	10.7	2
15	Ireland	Wexford	52^{o}16'N	6^{o}30'W	54	150	5	1033	10.1	3
21	Netherlands	Wageningen	51^{o}58'N	5^{o}40'E	7	0,108,108	5	760	9.6	3
10	Germany	Renningen	48^{o}46’N	9^{o}11’E	460	150	4,5,5	693	8.2	3
11	Germany	St. Johann	48^{o}28'N	9^{o}18E	700	150	4	1046	7.4	2
9	France	Auzeville Tolosane	43^{o}05'N	1^{o}43'E	162	120	3,2,3	680	13.0	3
34	Switzerland	Zurich-Reckenholz**	47^{o}26'N	8^{o}32'E	491	150	5	1031	9.4	3
24	Norway	Ås	59^{o}40’N	10^{o}51’E	95	135	3	785	5.3	3
35	Wales	Aberystwyth	52^{o}26'N	4^{o}01'W	30	90	4	1038	9.7	3
43	Ireland	Athenry	53^{o}17'N	8^{o}44’W	40	75	7	885	10.4	2
36	Wales	Bronydd Mawr	51^{o}57'N	3^{o}37'W	323	93	4,3,4	1500	8.2	3
27	Poland	Brody	52^{o}26'N	16^{o}18'E	91.4	90	4,3	587	8.0	2
44	Ireland	Moorepark	52^{o}08'N	8^{o}16'W	48	100	7	1207	9.5	2
26	Poland	Brody	52^{o}26'N	16^{o}18'E	94.2	120	4,4,3	587	8.0	3
22	Norway	Saerheim	58^{o}46'N	5^{o}39'E	90	0	3	1180	7.1	3
33	Sweden	Öjebyn (Piteå)	65^{o}19'N	21^{o}24'E	5	60	2,3,2	539	2.1	3
23	Norway	Tromsø	69^{o}40'N	18^{o}56'E	15	60	2	1031	3.1	3

19	Lithuania (b)	Dotnuva	55°24'N	23°50'E	71	120	3,2,2	650	6.1	3
7	Finland	Mikkeli***	61°40'N	27°13'E	107	60	3	643	3.1	2
25	Norway	Løken	61°07'N	9°04'E	435	80	2	576	1.6	3
18	Lithuania (a)	Dotnuva	55°24'N	23°50'E	71	120	3	650	6.1	3
30	Spain	Gosol	42°13'N	1°39'E	1410	0	2	948	7.9	1
16	Italy	Ottava	40°44'N	8°32'E	80	31, 57, 61	4,5,5	547	16.2	3
20	Lithuania (c)	Dotnuva	55°24'N	23°50'E	71	120	3,3,2	650	6.1	3
52	Canada	Lévis	46°46'N	71°12'W	43	60	2	1175	5.26	3
31	Sweden (a)	Svalöv	55°55'N	13°07'E	55	0	3	700	7.7	3
32	Sweden (b)	Svalöv	55°55'N	13°07'E	55	0	3	700	7.7	3
13	Iceland (a)	Korpa	64°09'N	21°45'W	35	40	2	900	4.5	3
14	Iceland (b)	Korpa	64°09'N	21°45'W	35	80	2	900	4.5	3
28	Spain	Zaragoza**	41°44'N	2°53'E	225	61	2,3,1	409	14.3	3

*Species codes as follows. Non-fixing grasses: Dg = *Dactylis glomerata*, Fa = *Festuca arundinaceum*, Lp = *Lolium perenne*, Lr = *Lolium rigidum*, Pp = *Phleum pratense,* Poa = *Poa pratensis.* N_2-fixing legumes: Mp = *Medicago polymorpha*, Ms = *Medicago sativa*, Ta = *Trifolium ambiguum*, Tp = *Trifolium pratense*, Tr = *Trifolium repens*.

**The plots at Switzerland were weeded for the first two harvests in the establishment year and the plots at Spain-Zaragoza were weeded only in year 1 of harvesting.

***Barley was used on all plots as a nurse crop at establishment and harvested in the establishment year, as per conventional practice at this site.