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Plant diversity in intensively managed grasslands can improve resource use efficiency and alleviate effects of extreme climate events

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Abstract
Ecological theory and empirical data demonstrate the ability of more diverse plant communities to better acquire available resources and convert them into aboveground biomass. This has obvious relevance for agricultural systems, and we have investigated the potential contribution of multi-species mixtures to intensive grassland systems. In the three-year multi-site Agrodiversity experiment, mixture yields were better than the best-performing monoculture, and mixtures were much more resistant than monocultures to weed invasion. Yield benefits established quickly (within the first year), persisted for at least three years, were robust for different relative proportions of the four species, and were evident across sites with very different soils, climatic conditions and yield potential. Total nitrogen (N) yield in the forage of grass-legume mixtures was significantly greater than for grass monocultures at a majority of evaluated sites at all three years. This N gain to mixtures increased with increasing legume proportion up to one third of legumes. Thus, across sites and years, mixtures with one-third proportion of legumes attained ~95% of the maximum N yield acquired by any stand and had 57% higher N yield than grass monocultures. Equally important, the relative N gain in mixture was not correlated to site productivity ($P = 0.500$), suggesting that, within climatic restrictions, balanced grass–legume mixtures can benefit from comparable relative gains in N yield across largely differing productivity levels. Multi-species mixtures offer considerable potential as an adaptation option to climate changes. In field experiments, mixtures better resisted the short-term effects of severe drought, and mixtures under drought yielded better than the average of monocultures under rainfed conditions.

Keywords: legumes, mixtures, yield, diversity, resource utilisation, drought

Introduction
Ecological research in species-rich and nutrient-poor systems shows that higher plant diversity increases the yield of aboveground biomass, but mixture yields generally did not exceed yields of the best monoculture (Cardinale *et al.*, 2007). The ability of more diverse plant communities to acquire available resources and convert them into above-ground biomass has obvious relevance for agricultural systems, although the results from extensively-managed (low-nutrient) semi-natural grasslands do not necessarily extrapolate to intensively-managed grasslands. Thus, our work has addressed the question: how can we improve the design of multi-species agricultural mixtures to improve yield efficiency and sustainable resource utilisation? The paucity of multi-species agronomic experiments with more than two species that have been conducted across multiple

sites, years and mixtures means that general predictions about the benefits of multi-species mixtures remain largely untested. Here, we provide an overview of our recent work on multi-species grassland mixtures. We present results from an international multi-site experiment that manipulated plant diversity within a forage grassland system. We conclude by presenting recent experimental evidence on the role of diversity in mitigating the impacts of experimental drought on grassland. This paper is largely based on research conducted as part of the Agrodiversity (Kirwan *et al*. 2014), MultiSward (www.multisward.eu) and AnimalChange (www.animalchange.eu) projects.

Plant diversity enhanced yield and reduce weed invasion in intensively managed grassland systems: the Agrodiversity experiment

The Agrodiversity experiment was conducted at each of 31 sites (17 European countries and Canada). Fifteen grassland communities comprising four monocultures and eleven mixtures of four functional types of species (each represented by one species) were sown at two seed density levels (see Finn *et al*., 2013 for details). The choice of species for use in multi-species mixtures can be strategically designed to include traits that maximise complementarity and interspecific interactions to improve resource utilisation and yield of above-ground biomass. Thus, we selected functional types that consisted of a fast-establishing grass, a fast-establishing legume, a temporally persistent grass and a temporally persistent legume. The selection of species varied across sites, but the most commonly used species were *Lolium perenne* L., *Trifolium pratense* L.*, Dactylis glomerata* L.*,* and *Trifolium repens* L. To investigate the sensitivity of yield responses to different relative proportions of the functional types, the sowing rate of the same four species was systematically varied to produce eleven mixture communities with four different initial levels of evenness (E) (see Kirwan *et al*., 2007): four mixtures dominated in turn by each species (sown proportions of 70% of one species, and 10% of each of the other three species, E = 0.64), six mixtures dominated in turn by pairs of species (40% of each of two species, and 10% of each of the other two, E = 0.88) and the community with equal proportions (25% of each species, E= 1). Across 31 sites, this design resulted in 30 experimental plots per site and a total of 930 plots.

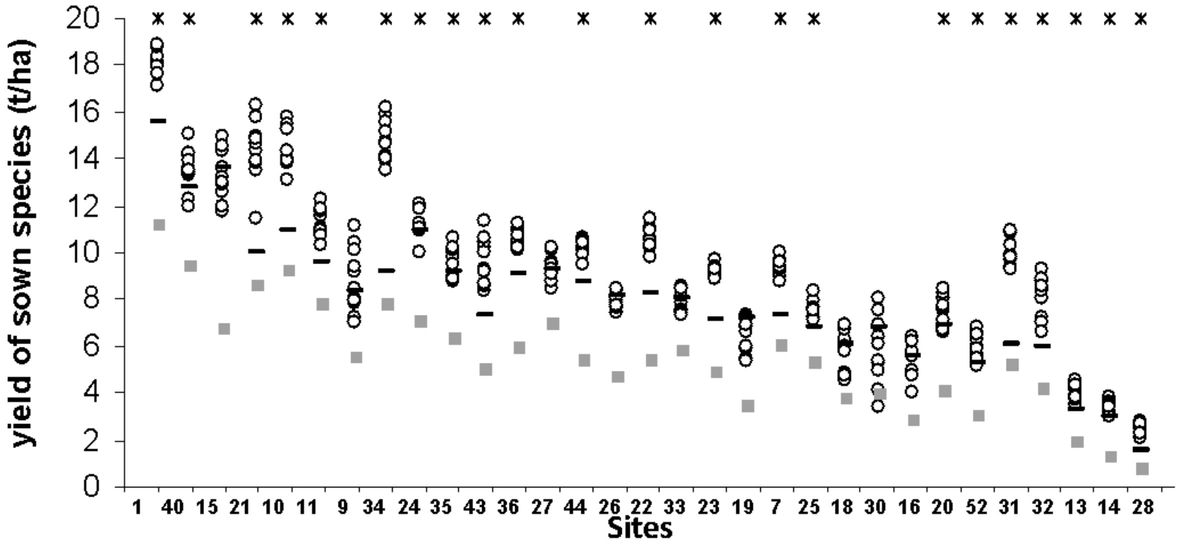

Fig. 1. Mixtures generally yielded more than the best monoculture. Average annual yield over the whole experimental duration of yield of sown agronomic species (excludes weeds) at each of 31 sites. Each open circle represents the average aboveground biomass for a specific mixture community across multiple years; horizontal lines represent the yield of the best-performing monoculture; shaded boxes represent the mean monoculture performance. Significant transgressive overyielding is indicated by an asterisk over a site at the top of the panel. From Finn *et al*. (2013). The site numbers correspond to individual experiments. Data available from Kirwan *et al.* (2014).

There was a considerable range in site productivity, reflecting the different geographical and climatic regions across the study sites. Annual averages of total yield (dry matter) per site ranged from about 18 t ha^{-1} $year^{-1}$ to about 3 t ha^{-1} $year^{-1}$. Across all sites, yield of sown species (averaged across years and seed density) of mixtures exceeded that of the mean monoculture in 99.7% of mixture communities with an average (across all mixtures and sites) ratio of mixture/monoculture yield of 1.77 (Fig. 1). Transgressive overyielding (better yields than in the best monoculture) occurred in 79% of mixture communities and was significant at 71% of sites. At sixteen sites, all of the mixture communities yielded more than the best monoculture community. The yield benefit of mixtures was already evident in year 1, and persisted for the three years of the experiment. Across all sites, monocultures displayed much higher levels (and variability) of weed invasion than mixtures. The median percentage of weed biomass in the total yield of monocultures increased over time (15% in year 1, 20% in year 2 and 32% in year 3); in contrast, the median percentage of weed biomass in the mixtures remained consistently low (4% in year 1, 3% in year 2 and 3% in year 3).

What is most striking about these results is that the magnitude of mixture benefits was sufficient for mixtures to regularly yield more than the best-performing agronomic monoculture. This is striking for two main reasons: first, the rapidity and frequency of transgressive overyielding and second, the occurrence of transgressive overyielding across such a wide range of variation in the evenness of the mixtures.
The *a priori* design of the mixtures intentionally included a combination of traits related both to nitrogen (N) acquisition and temporal development, and we attribute the observed yield benefits to the resource complementarity arising from the interactions of these functional traits. Only synergistic interactions can produce transgressive overyielding (as opposed to selection effects), and transgressive overyielding is only achieved when the net effects of interspecific interactions are sufficiently strong (Kirwan *et al*., 2007, 2009). The combination of grasses and legumes is well known to result in mixture benefits due to the transfer of symbiotically fixed nitrogen from legumes to grasses (e.g. Boller & Nösberger, 1987; Nyfeler *et al*., 2009, 2011).

Grass-legume mixtures maintained yield despite substantial reductions in N fertiliser

Grass-legume mixtures in grassland forage systems can benefit from symbiotic N_2 fixation of legumes, thereby increasing total harvest yield, total N yield (N_{tot}) and forage quality. Because legumes have access to atmospheric N_2 for their N requirements, the relative availability of soil N increases for grasses in mixtures due to 'N sparing' (increased availability of soil N because legumes rely on symbiotic N_2 fixation). Therefore, the use of grass-legume mixtures could allow substantial reductions in amounts of industrial N fertilisers in agricultural grassland systems without a compromise in yield. At the Swiss site of the Agrodiversity experiment, Nyfeler *et al.* (2009) compared monoculture and mixture yields across three levels of nitrogen (50, 150 and 450 kg N ha^{-1} $year^{-1}$). Their results indicated a high potential for N-fertilizer replacement: grass–clover mixtures containing 40–60% clover and receiving 50 or 150 kg N ha^{-1} $year^{-1}$ achieved the same yield as grass monocultures fertilized with 450 kg N ha-1 year-1 (Nyfeler *et al*., 2009). Diversity–productivity effects were reduced at the highest level of N fertilization and at 450 kg N ha^{-1} $year^{-1}$, they virtually disappeared in the third year.

Plant diversity enhanced total nitrogen yield in intensively managed grassland systems: the Agrodiversity experiment

More efficient use of N is a major global challenge for the sustainable intensification of agricultural systems, and there is considerable potential for N self-sufficiency to be gained through home-grown N_2-fixing crops, as well as a reduction in the negative environmental effects associated with the manufacture and use of N fertiliser. As part of the Agrodiversity experiment, we analysed the total N yield (N_{tot}) in forage, the realized legume proportion of swards, and the potential N yield gain in mixtures as compared to grass monocultures ($N_{gainmix}$) (Suter *et al*. 2015).

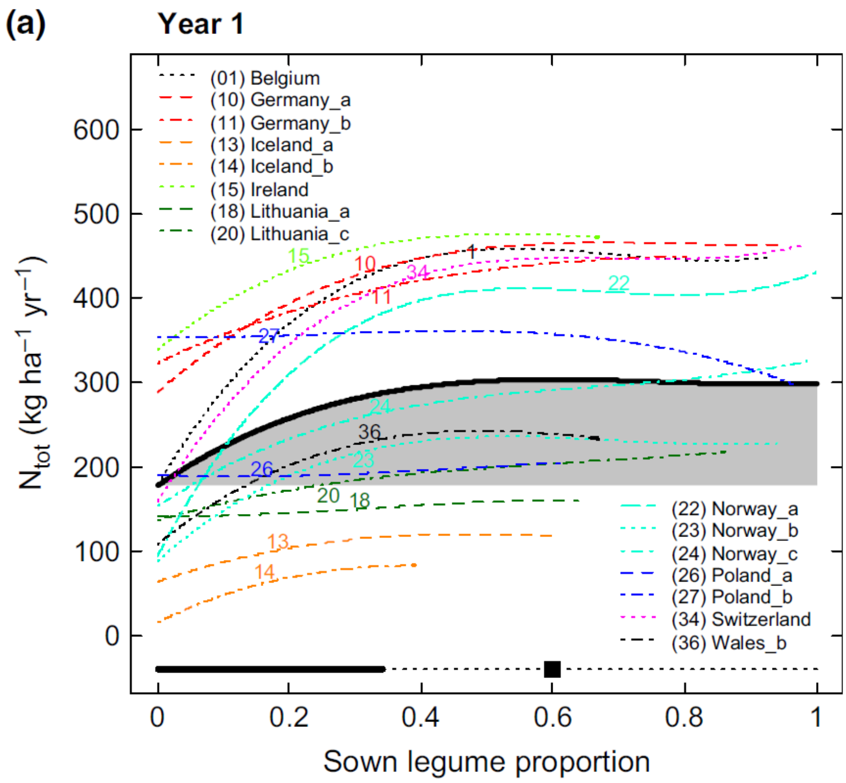

Fig. 2. Total nitrogen yield (N_{tot}) as affected by legume proportion in grassland mixtures. The bold black line displays the predicted N_{tot} across all sites, and colored lines display predicted N_{tot} for individual sites over the range of legume proportion in year 1. The vertical bold red line indicates the predicted N_{tot} advantage (relative to the grass-only sward) for a mixture with a grass:legume ratio of ⅓:⅔. At this point, about 95% of maximum N_{tot} was gained, which is about 55% greater than that in the average grass monoculture. Numbers on lines refer to sites and are located at the mean legume proportion realized across all stands per site. The shaded area indicates N yield gain in mixture as compared to grass monocultures across sites. The horizontal bold line at the bottom indicates the range of legume proportion for which N_{tot} across all sites was significantly smaller ($P \leq 0.05$) than at maximum (■). From Suter *et al*. (2015).

The amount of N_{tot} of mixtures was significantly greater ($P \leq 0.05$) than that of grass monocultures at the majority of evaluated sites in all three years (Fig. 2). N_{tot} and thus $N_{gainmix}$ increased with increasing legume proportion up to one third of legumes. With higher legume percentages, N_{tot} and $N_{gainmix}$ did not continue to increase (see vertical bold red line in Fig. 2). Thus, across sites and years, mixtures with one third proportion of legumes attained ~95% of the maximum N_{tot} acquired by any stand and on average had 57% higher N_{tot} than grass monocultures. The relative N gain in mixture ($N_{gainmix}/N_{totmix}$) was not correlated to site productivity ($P = 0.500$), suggesting that, within climatic restrictions, balanced grass–legume mixtures can benefit from comparable relative gains in N yield across a large productivity gradient. Thus, although N_{tot} in mixture was strongly affected by site productivity ($P = 0.003$), the relative N yield gain in mixture was not (Suter *et al*. 2015). This was a most important result of the study, because it means that less productive sites as well as more productive sites can equally profit from grass–legume mixtures to increase relative N output. We conclude that grass-legume mixtures can improve N use efficiency of productive agricultural grassland systems under a wide range of environmental conditions.

Yield benefits of mixtures persist under grazing

Many experiments on multi-species mixtures have been conducted under conditions where the forage has been harvested by mowing. An important question is whether the benefits of mixtures that are observed under mowing also prevail under grazing. There are many experiments with grass-clover mixtures under grazing;

however, comparisons of more species-rich combinations are less common. A recent study investigated whether grazing modifies the benefits of mixtures on total N yield compared to mowing (Huguenin-Elie *et al*., 2016). The design included N_2 fixing and non-fixing species, as well as shallow- and deep-rooting species. *Lolium perenne* (Lp) monoculture and mixtures with *Cichorium intybus* (Ci), or/and *Trifolium repens* (Tr) and *Trifolium pratense* (Tp) were compared under grazing or mowing for their N yield and capture of fertilizer and atmospheric N_2. Mixtures of the N_2 fixing and the non-fixing species with 145 kg N ha^{-1} yr^{-1} yielded as much N as the *L. perenne* monoculture fertilized with 350 kg N ha^{-1} yr^{-1}, showing the tremendous benefit of mixtures for N efficiency (Table 1). The benefits of the mixtures on N yield were similar under grazing and mowing. Grazing did not modify the proportion of N derived from fertilizer and symbiotic N_2 fixation in the plants.

Table 1. Effect of sward types on dry matter and total nitrogen yield. Reproduced from Huguenin-Elie *et al*. (2016).

Sward type	**N fertiliser (kg N ha^{-1} yr^{-1})**	**Dry matter yield (kg ha^{-1} yr^{-1})**	**Total N yield (kg ha^{-1} yr^{-1})**
Monoculture Lp	145	4.7a	118a
Mixture LpCi	145	5.7b	142a
Mixture LpTrTp	145	6.8c	206b
Mixture LpCiTrTp	145	7.0c	213b
Monoculture Lp 350N	350	6.7c	225b
Standard error of the mean		0.4	13
P-value ANOVA			
Sward type		<0.05	<0.01
Management type		<0.01	<0.01
Interaction		0.88	0.67

In a two-year grazing experiment (with 75 kg N ha^{-1} yr^{-1}) in France, an increase of botanical complexity from one to five species (two grasses, two clovers and chicory) resulted in positive effects on animal performance (Roca-Fernández *et al*., in press). They distinguished between monocultures of perennial ryegrass, 'mixed swards' of grass and clover, and 'multi-species swards' of grasses, clovers and chicory. Compared to mixed swards, multi-species swards improved production of milk (+0.8 kg/day) and milk solids (+0.04 kg/day), which was attributed to enhanced sward quality and increased dry matter intake (+1.5 kg DM/day).

In a two-year grazing experiment in the northeastern USA (no nitrogen fertiliser was applied), four mixture communities were compared: two species (one grass, one legume), three species (one grass, one legume, and chicory), six species (three grasses, two legumes and chicory), and nine species (four grasses, four legumes and chicory). In a dry year, the two-species mixture (4800 kg ha^{-1} dry matter) yielded less than the other mixtures (7600 kg ha^{-1} dry matter); there was no difference in dry matter yields (9800 kg ha^{-1} dry matter) in the year with plentiful rainfall (Sanderson *et al*. 2005).

Both of these experiments point both to the ability of more complex (>2 species) mixtures to improve yields. In addition to the work of Roca-Fernández *et al*., (in press), several studies review the contribution of grass-legume mixtures to the nutrition and production of livestock (e.g. Lüscher *et al*. 2014, Dewhurst *et al*. 2009) The Sanderson *et al*. (2005) study also suggests the ability of more complex mixtures to mitigate the effects of adverse weather conditions under grazed conditions.

Yield benefits of mixtures persisted under experimental drought

Climate change is predicted to result in increased climate variability, and an increase in severe weather events. The combined effects of increased variability in precipitation and the amount of precipitation per event, e.g.

prolonged periods of drought or waterlogging can result in reduced yields in grassland systems. Although grass-legume combinations consistently yielded more than predicted from monoculture yields (Finn *et al*. 2013, above); there have been relatively few tests of whether such an advantage of multi-species mixtures remains evident under environmental stress, e.g. drought conditions. Although we can expect a short-term effect of drought on yields, key knowledge gaps also remain about the extent to which annual yields of intensively managed temperate grasslands are affected.

Fig. 3. Overhead view of the rainout shelters at the Wexford site.

We selected the following four forage species based on the factorial combination of their specific functional traits related to rooting depth and manner of N acquisition: a shallow-rooted non-legume (*Lolium perenne* L.), a deep-rooted non-legume (*Cichorium intybus* L.), a shallow-rooted legume (*Trifolium repens* L.), and a deep-rooted legume (*Trifolium pratense* L.). Monocultures and mixtures were sown with systematically varying proportions of these four species, and were established as control treatment under ambient rainfed conditions and as drought treatment in which a summer drought event of nine weeks was simulated at both sites using rainout shelters (see Hofer *et al*., 2016a for details).

The experimental drought induced severe stress at Tänikon and Reckenholz and extreme stress at Wexford, and the short-term effects of drought reduced an individual harvest by 48% at Reckenholz, and 85% at Wexford (Fig. 4). The yield advantage from mixing species could compensate for this short-term drought impairment under severe drought at Tänikon and Reckenholz. The four-species mixtures under severe drought stress produced higher yield under drought than monocultures under rainfed conditions (Fig. 4a,b), but not under extreme drought stress (Fig. 4c).

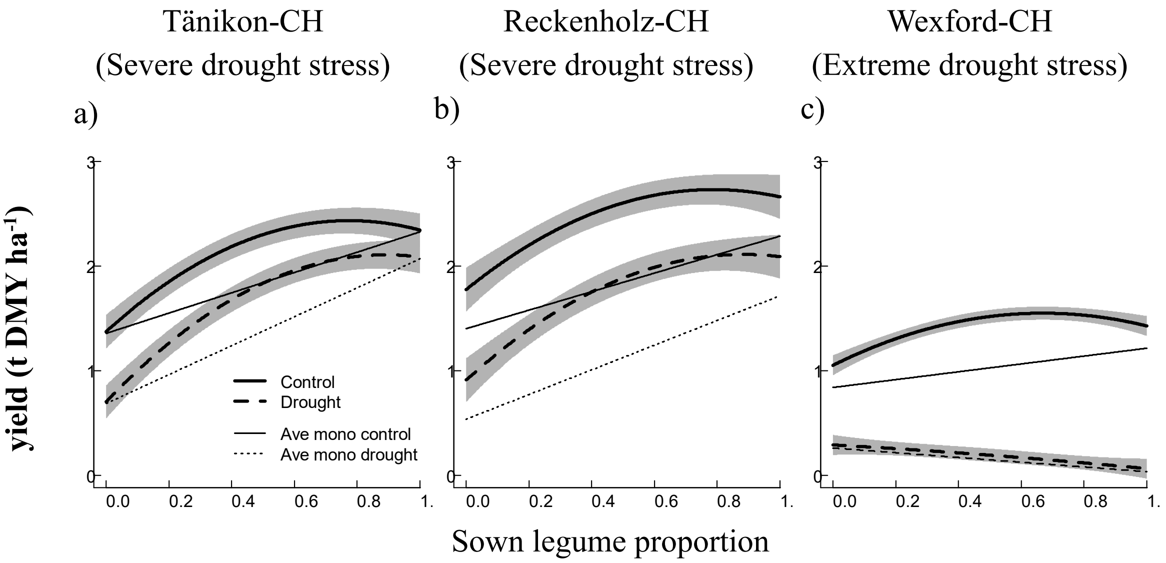

Fig. 4. Four-species mixtures under severe drought stress produced higher yield under drought than the average of the four monocultures under rainfed conditions. These graphs show predicted aboveground biomass yield of the second regrowth during the drought period for increasing legume proportions under rainfed control (bold solid line) and drought conditions (bold dashed line) at Tänikon-CH (a), Reckenholz-CH (b) and Wexford-IE (c). Predicted lines (±1 SE, grey shaded) are based on regression analysis and are displayed for mixtures that are equally composed of the two non-legumes and legumes, meaning that the left and right endpoints of lines represent binary mixtures of the two grass and the two legume species, respectively, and the prediction at 50% legume proportion represents the equi-proportional mixture. Thin lines represent aboveground biomass yield that could be expected from the weighted average of the respective monocultures in the absence of any diversity effect. DMY: dry matter yield. From Hofer *et al*. (2016a).

In a separate analysis of yield aggregated across harvests, the total annual yield of mixtures under drought exceeded the average annual yield of the rainfed monocultures (Table 1). For annual yield, the benefit due to mixtures (33% increase) was substantially greater in magnitude than the impact of drought (5% reduction) in our experiment. These results illustrate the high potential for multi-species mixtures to compensate for drought-induced yield losses.

Table 2. Annual aboveground biomass yield (kg DM ha^{-1} $year^{-1}$, mean ±1 SE, n = 3) of the four species in monoculture, the average of these four species, the equi-proportional mixture of the four species and the mixture benefit under rainfed control and drought conditions at Reckenholz and Wexford. Also shown is the percentage change of annual yield (% CAB) due to drought. Statistical inference is based on the natural log transformed data. From Hofer *et al*. (2016b).

	Reckenholz				**Wexford**			
	Control kg ha^{-1} yr^{-1}	**Drought** kg ha^{-1} yr^{-1}	**%CAB**	***P*-value**	**Control** kg ha^{-1} yr^{-1}	**Drought** kg ha^{-1} yr^{-1}	**%CAB**	***P*-value**
L. perenne	8680	8632	-1	0.860	9571	9248	-3	0.804
C. intybus	10121	8891	-12	0.003	8227	8490	+3	0.943
T. repens	11370	10671	-6	0.090	9051	8573	-5	0.599
T. pratense	16091	15448	-4	0.240	14218	12683	-11	0.260
Mean mono	11566	10911			10267	9749		
Mixture	16346	14814	-9	0.014	13187	12537	-5	0.604
Mixture benefit	4780	3903			2920	2788		

Over short durations, the yield advantage from mixing species could compensate for yields being strongly affected by drought. Over the whole year, the strong overyielding of mixtures resulted in them exceeding the average yield of the rainfed monocultures under severe drought conditions (Hofer *et al.* 2016b).

These results illustrate the nature and extent of drought in intensively managed grassland systems. At the scale of individual harvests, there can be severe effects of drought on yield, although these are probably mediated by the pre-drought levels of soil moisture, as well as the moisture retention properties of the soils once drought is underway. At the scale of total annual yield, however, the yield benefit due to mixtures (33% increase in annual yield) can be substantially greater than the effect of drought (5% reduction in annual yield) in our experiment. We found a relatively rapid recovery when soil water supply increased after the drought, even after the extreme drought stress at the site in Wexford. Such post-drought reaction might be explained by our application of N fertilisers in equal amounts to both the rainfed control and the drought treatment. During the drought, much of this N was not available, and the recovery of post-drought yields was likely boosted by the increased availability of this N in the communities subjected to the drought treatment (Hofer *et al*. 2016ab).

Conclusions

Overall, our results indicate that the choice of species for use in multi-species mixtures can be strategically designed to include several traits (and not just traits for symbiotic N_2 fixation) that maximise complementarity and interspecific interactions to improve resource utilisation and yield of aboveground biomass. In the three-year multi-site Agrodiversity experiment, mixture yields were sufficiently high to result in transgressive overyielding, and mixtures were much more resistant than monocultures to weed invasion. Yield benefits established quickly (within the first year), persisted for at least three years, were robust for different relative proportions of the four species, and were evident across sites with very different soils and climatic conditions (Finn *et al.* 2013).

Using mixed swards instead of pure grass stands, more N yield can be expected for a given amount of N fertilizer applied (Suter *et al.* 2015). Alternatively, if the aim was to reduce fertilizer N application for financial, regulatory, and/or environmental reasons (Lüscher *et al.*, 2014), our data show the potential to do so without necessarily compromising N yield and total harvested biomass (Nyfeler *et al.*, 2009; Finn *et al.*, 2013). In the face of high economic and environmental costs of industrial N, the contribution of symbiotic N2 fixation by legumes to grassland N supply appears to be a key strategy to maintain and increase current levels of production and protein self-sufficiency in a more sustainable way (Lüscher *et al.* 2014). Multi-species mixtures offer considerable potential as an adaptation option to climate changes (Hofer *et al.* 2016).

We conclude that legume-based mixtures can contribute to more sustainable livestock systems. Looking to the future, there is a need for further knowledge of grazing management for farming systems based on multi-species grasslands, and for further work on the quality and quantity of intake and quality of animal output.

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