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Effect of plant diversity and drought on the agronomic performance of intensively managed grassland communities

This thesis is submitted to Trinity College Dublin in fulfilment of the requirements for the degree of Doctor of Philosophy by

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Guylain Grange

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Summary

Temperate agro-ecosystems are crucial for food production and financially important for the rural economy, but can have strong environmental impacts and are threatened by increased frequency of extreme weather events. Over the last decades, research showed that plant diversity can enhance ecosystem productivity and resilience in temperate agro-ecosystems. However, consistent knowledge gaps remain in the characterization of the biodiversity – ecosystem functioning relationship (BEF). In particular, more work is needed on the potential transfer of ecological principles about BEF relationship to intensively managed agro-ecosystems. Better knowledge of the effect of plant diversity on field scale productivity, resilience and ecosystem services could help to enhance agronomic and environmental performances of intensive agro-ecosystems.

To investigate the effect of plant diversity on ecosystem functioning, I carried out a grassland experiment in Wexford, Ireland. The field was sown using a six-species pool from three functional groups: grass (*Lolium perenne* and *Phleum pratense*), legume (*Trifolium pratense* and *Trifolium repens*) and herb (*Cichorium intybus* and *Plantago lanceolata*). Plant diversity was manipulated using a simplex design to form 19 communities of one to six species, that were established across a total of 39 plots, all receiving an annual fertiliser rate of 150 kg N ha-1 yr-1 (150N). Four extra replicates of *L. perenne* receiving double nitrogen fertiliser were added (300N). Each plot was randomly subdivided in two different water supply treatments: one subplot was a control (rainfed), and a two-month summer drought was simulated on the other (drought). Subplots were harvested seven times each year for two years. Grassland cover was then terminated to establish a model crop, keeping the same field layout. No drought was applied, and all plots received 40N, including the former 300N plots. Four crop harvests were performed to measure the legacy effect of the previous grassland treatments. A series of analyses were carried out on the data from across the three years of data using Diversity-Interactions modelling to relate ecosystem functions to plant diversity (including species identities and interactions) and climatic conditions (water supply).

Biomass yield in the grassland phase of the rotation was strongly influenced by plant diversity. Interactions between functional groups enhanced annual biomass production to the point that a wide range of mixtures outperformed the best monoculture (i.e. transgressive overyielding). Interactions were maintained under drought conditions. I compared the 300N *L. perenne* monoculture (low-diversity, high-input) to the 150N equi-proportional six-species mixture (high-diversity, low-input). Even when stressed by drought, the high-diversity, low-input mixture outperformed the rainfed low-diversity, high-input comparison.

No evidence of grassland species interactions were found on the legacy effect, but strong dissimilarities were observed among species. Higher legume proportion in grassland led to higher legacy effect. The lowest legacy effect was from the 300N *L. perenne* monoculture. Due to the absence of interspecific interactions on legacy effect, no overyielding was observed in the crop phase of the rotation. Drought applied in the grassland phase had a constant effect on legacy, reducing crop yield by the same magnitude across all plots. Considering the two phases of the grassland-crop rotation, selection of the 'best' grassland community depends on the relative importance of each phase of the rotation. High diversity favoured grassland phase yield, while high legume content stimulated the yield of a follow-on crop receiving 40N. A diverse grassland mixture including a high proportion of legume would deliver high performances in both phases.

To upscale the analysis of the BEF relationship, I investigated multiple ecosystem functions that reflect the multiple purposes of intensive agro-ecosystems. The effects of diversity on dry matter yield, legacy effect, nitrogen fertiliser efficiency, yield consistency, forage digestibility and weed suppression were simultaneously assessed in a multifunctionality analysis. To do so, a multivariate model was combined with the Diversity-Interactions approach. The effect of plant diversity was function-specific. Species interactions were strong and positive for half of the functions, and neutral for the others, with the overall effect of diversity remaining positive. The benefit of interactions added to divergent species identity effects across functional groups resulted in species rich balanced mixtures achieving the highest performance averaged across functions. The low-diversity, high-input comparison was amongst the lowest performing communities. Again, the effect of drought was either constant or applied only to species identities, depending on the function. Across functions, balanced mixtures of grass, legume and herb outperformed both the best monocultures and the low-diversity, high-input comparison, while mitigating the effects of an extreme summer drought.

This thesis demonstrates that averaged across functions, interactions were strong enough for a wide range of mixtures to achieve transgressive over-performance under intensive management, even when a summer drought occurs. Although species interactions were not significant for all functions, no negative interaction effect was noted and the net effect of species interactions was positive. Drought did not affect the strength of plant interaction effects averaged across functions, resulting in a relatively higher benefit of diversity when species identity effects were reduced by drought stress. Positive interactions, even under drought conditions, add to the overall benefit of plant diversity in intensively managed agro-ecosystems, in contrast with increased fertiliser that showed poor performances across most of the functions studied.

In conclusion, I show that mixing plant species is a practical way of enhancing the performances of intensively managed agro-ecosystems in several ways across a crop rotation, while mitigating the effect of a weather disturbance. Intensification based on ecological principles proved to be more efficient than increased fertiliser use on most of the aspects studied. Multi-species systems are thus a promising tool to reduce the reliance on fertiliser inputs and to help protect against extreme weather conditions.

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L’utopie a changé de camp:
est aujourd’hui utopiste celui qui croit que tout peut continuer comme avant.

Pablo Servigne

Publications

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Chapter 1

Introduction

The global human population is expected to reach 10 billion people by 2050, and is highly reliant on agriculture to produce food in sufficient quantity and quality. To do so, over 50% of earth's ice-free land is used for farming (Shukla *et al.*, 2019). Since the Green Revolution in the 1950's, there has been a consistent intensification of agricultural production, through increased use of inputs such as chemical fertiliser. In the meantime, global climate change is threatening food production by an increase in severe weather events, including changes in frequency and magnitude of summer drought, winter precipitation and temperature fluctuations in temperate areas (Hoegh-Guldberg *et al.*, 2018).

The impact of extreme weather events increases with higher management intensity of agricultural systems (Vogel *et al.*, 2012; Finger *et al.*, 2013). Intensively managed agro-ecosystems are characterised by reliance on low biodiversity and high levels of inputs with a strong focus on food production (Fig. 1.1). Management intensity is however facing challenges (Fig. 1.2), as it is associated with practices (e.g. increased use of fertiliser) that amplify climate change processes through higher release of gas with high greenhouse potential (Stocker *et al.*, 2013). In addition, intensively managed systems rely on lower plant diversity (Karp *et al.*, 2012), which reduces the range of ecosystem functions delivered, and resilience to weather events (Isbell *et al.*, 2015; Dardonville *et al.*, 2020; Schils *et al.*, 2022 and see Fig. 1.2). The re-introduction of diversity at farm scale appears as a way to stimulate agro-ecosystems productivity and stability (Isbell *et al.*, 2017), helping to address some environmental concerns. The aim of this thesis is to explore how principles from the relationship between biodiversity and ecosystem function can inform the designed inclusion of plant diversity as a practical management tool for enhancing the agro-environmental performance of intensively managed grassland systems (Figure 1.2). The primary hypothesis is whether increased plant diversity can increase the productivity of intensively managed grasslands, as well as their longer-term effect in a rotation. I also investigate how plant diversity can promote multifunctionality and resilience when subjected to a weather disturbance.

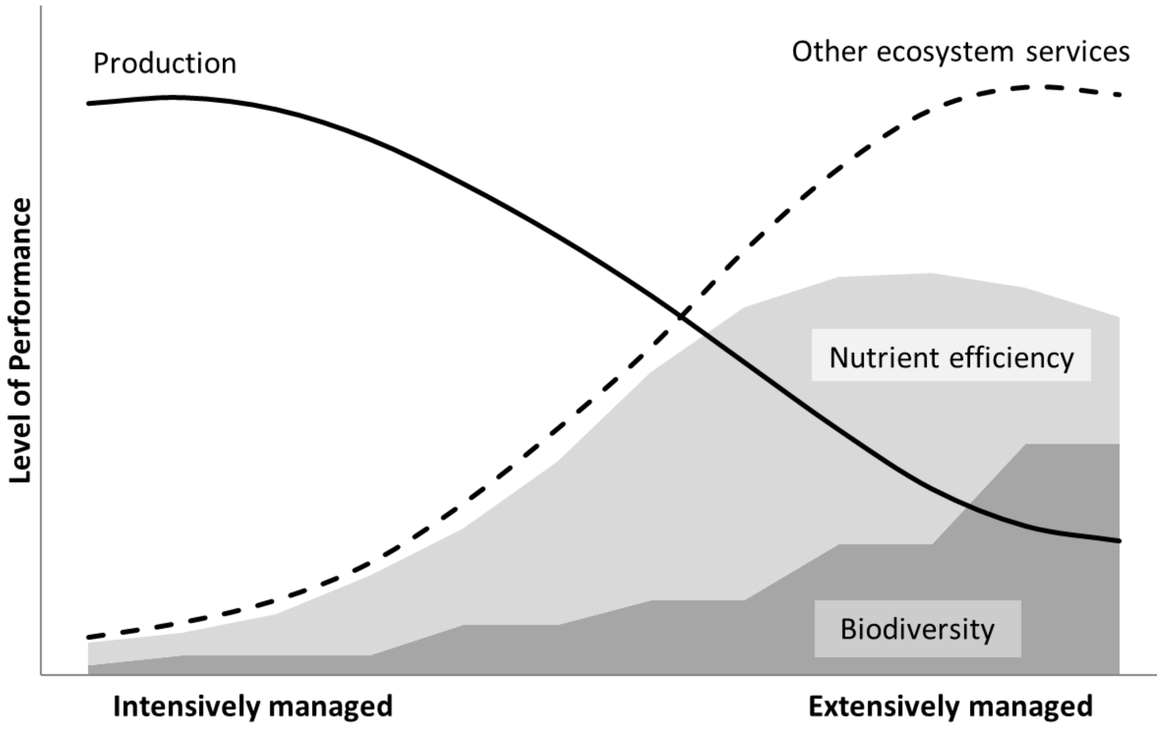


Figure 1.1. Conceptual illustration of the evolution of farm agro-environmental characteristics per unit area, along a gradient of management intensity (adapted from Delaby *et al.* (2020)). It highlights contrasting attributes along a gradient from natural or extensive systems to intensive systems.

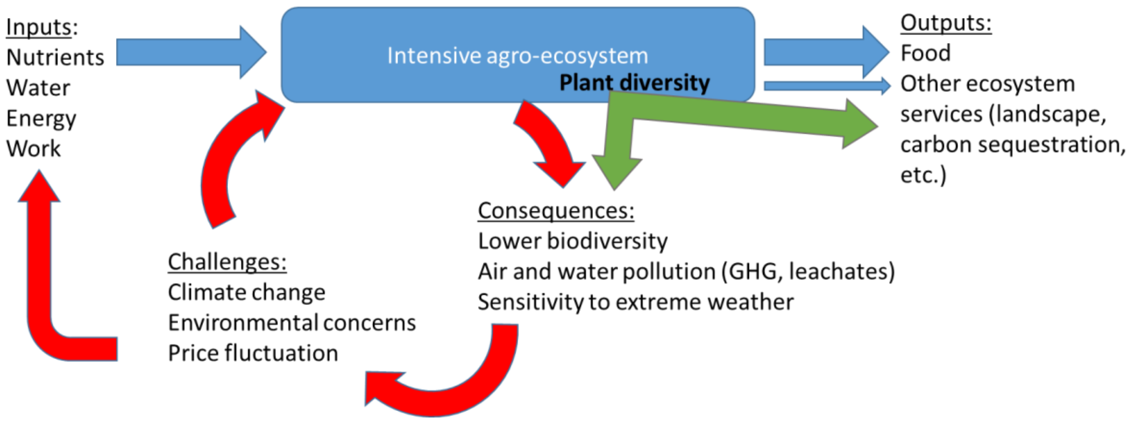


Figure 1.2. Summary of process (blue arrows) and challenges associated with intensive agriculture. This concept map illustrates the concerns (red arrows) associated with intensively managed agro-ecosystems. It also highlights the overall goal of the work presented here, which is to identify if increasing plant diversity can help to promote intensive agro-ecosystems services and reduce negative consequences of the food production process (green arrows).

Diversity effects on ecosystem functioning: the scientific basis

This section gives a brief overview of the relationship between biodiversity and ecosystem function (BEF). After characterizing the BEF relationship principles, I discuss their utilization for promoting functioning and resilience of intensively managed agro-ecosystems.

Ecological principles from natural grasslands suggest that higher diversity enhances functioning

The link between biodiversity and ecosystem functioning has long been an important topic for ecologists. In particular the effect of biodiversity on the quantity and stability of biomass production has been studied in depth in natural grasslands (Cardinale *et al.*, 2007; Craven *et al.*, 2016; Isbell *et al.*, 2017). Many experiments were performed on experimentally simulated natural grassland communities to disentangle the processes involved in this biodiversity – ecosystem functioning relationship (BEF). They confirmed that plant diversity could enhance grassland services delivered, across a large panel of ecosystem functions such as productivity, stability and resilience due to BEF (Vogel *et al.*, 2012; Gross *et al.*, 2014; Isbell *et al.*, 2015; Grace *et al.*, 2016). However, most of these works only used richness as a predictor, and not any other metric for diversity. As pointed by Craven *et al.* (2018), diversity is multi-faceted, and its effect only partially captured by the single metric of the ‘number of species’ (i.e. species richness) which does not address composition, relative abundance nor evenness of species in the community.

Detailed exploration of the BEF relationship requires precise knowledge of the diversity, because the metric chosen could affect conclusions. Richness inherently attributes the same weighting to each species in a community, independently of their relative abundance or stronger impact on a response. This can create bias in case of dominance or keystone species in a community (Polley *et al.*, 2007; Mouillot *et al.*, 2011; Bessler *et al.*, 2012). Indeed, the multifaceted nature of diversity challenges the assessment of the BEF relationship. As an example, Figures 1.3 and 1.4 illustrate that species’ relative abundance can greatly influence the delivery of an ecosystem function, even while species richness is unchanged (see O'Hea *et al.*, 2010 for experimental results on this aspect). In species-rich ecosystems, integrating other aspects of diversity in BEF analysis can be challenging, because of the high number of species. Ways around specific characterization often require a deeper quantification of species functional traits, abundance, interactions or specific contribution to ecosystem functions (Loreau and Hector, 2001; Roscher *et al.*, 2007; Brophy *et al.*, 2017a; Craven *et al.*, 2018).

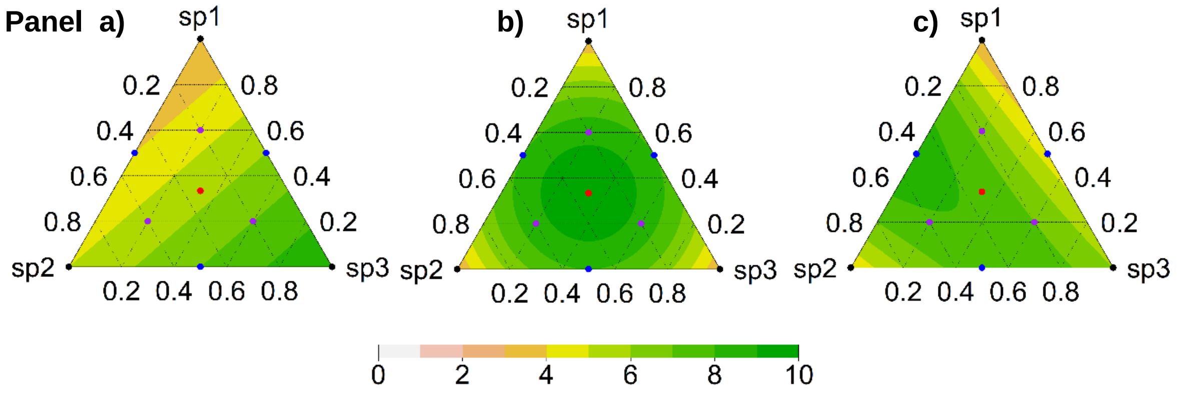


Figure 1.3 Ternary diagrams displaying the communities for a theoretical distribution of responses from communities built from a three species pool. Panel (a) shows a response with different species identity effects (sp1= 3, sp2 = 5 and sp3 = 9), but no interspecific interaction effects between each pair of species. Thus, the predicted performance of any mixture of the three species is a linear combination of their identity effects. Panel (b) shows a response with no difference among species identity effects (all = 3), and equal and strong interspecific interaction effects among each pair of species. Panel (c) shows a response with different identity (sp1 < sp2 < sp3) and interaction effects (sp1*sp2 > sp2*sp3 > sp3*sp1) among each pair of species. See paragraph "*Diversity-Interactions modelling approach and implications for experimental design"* for more details on the building of the response shape from species identity and interaction effects.

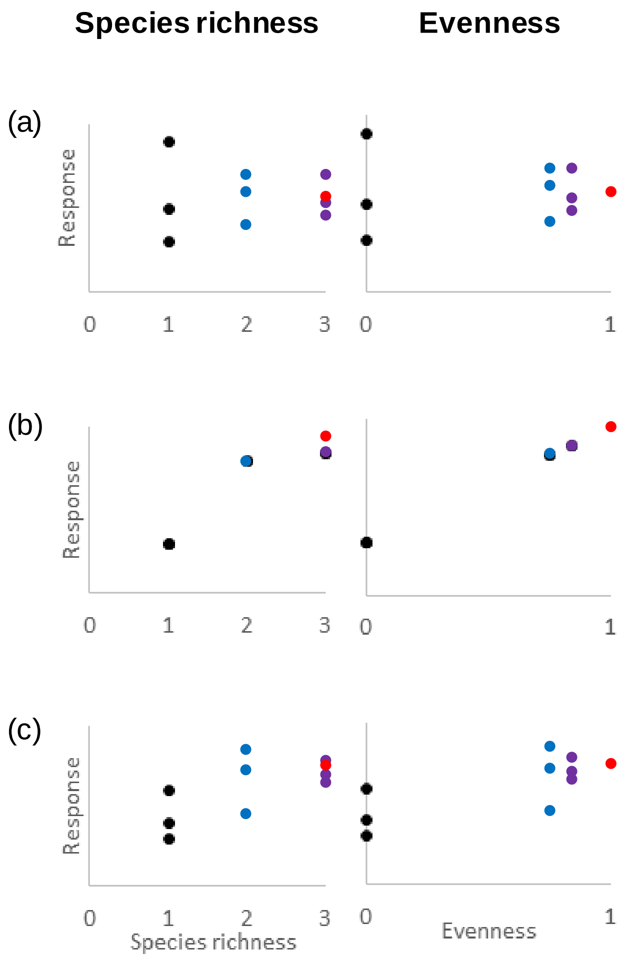


Figure 1.4. This set of graphs shows the response values for the coloured points displayed in the panels in Fig. 1.3, depending on the corresponding species richness and evenness. It illustrates how different patterns of species identities and interactions influence the spread and slope across diversity metrics (referring to panels a, b and c in Fig. 1.3). Also, it points to the amount of information lost when focusing on species identity and evenness, especially for low-richness communities (e.g. large unexplained variance for panels (a) and (c)).

Transferring principles from research on biodiversity and ecosystem function to intensively managed grasslands

Plant communities for intensively managed agriculture are generally of low species richness, and are established using a recommended species pool. Monocultures are common, due to genetic selection of species best adapted to intensive management (Matson *et al.*, 1997), and ease of management. The selection of adapted and high performing species can be combined with plant complementarity to enhance community functioning. Complementarity relates to how species synergistically use different resources or benefit from each other, and is easier to quantify in communities counting a small number of species, as it is generally the case in intensively managed grasslands (Loreau and Hector, 2001).

Research on extensively managed plant communities showed that complementarity between species of the same functional group can enhance productivity (Van Ruijven and Berendse, 2003). This work was confirmed by Gross *et al.* (2007) who showed that functional diversity could be sufficient to promote complementarity, within mixtures of grass species. Marquard *et al.* (2009) explored interactions between species and found that they could lead to transgressive overyielding. The occurrence of transgressive overyielding was defined by Trenbath (1974) as the occurrence of a mixture outperforming the best monoculture. For Marquard *et al.* (2009) and Cadotte (2013), functional distance between components of a mixture would enhance complementarity and thus promote chances of transgressive overyielding to occur.

In intensively managed grasslands, productivity remains the principal objective. Thus, the targeted communities are the most productive, but often selected among monocultures. Transgressive overyielding occurrence proves that diversity can lift the ‘maximum threshold’ associated with monoculture crops. This is what Finn *et al.* (2013) showed mixing four species with contrasting traits in a multi-site experiment under intensive management. They found transgressive overyielding in 60% of the 31 sites across a large climatic gradient in Europe. Species with contrasted traits were chosen in order to maximize complementarity and thus performance. Nyfeler *et al.* (2009) explored how designed plant interactions could promote intensive grassland resource efficiency by crossing diversity with levels of fertiliser inputs in an experiment. Due to grass-legume synergistic interactions, they found that mixtures receiving 50 kg N ha^{-1} yr^{-1} could yield as much biomass as the monocultures receiving 450 kg N ha^{-1} yr^{-1} by manipulating not just richness, but also relative abundance of functional groups with strong complementarity.

Designed plant communities with positive interactions are largely used in agro-ecosystems to promote ecological intensification, i.e. relying on ecological processes rather than increased input use to stimulate productivity (Tittonell, 2014). Indeed, as reviewed by Luscher *et al.* (2014), intensive and semi-intensive temperate grassland systems often rely on legume by non-legume interaction. However, plant interaction effects were often characterised in communities with limited number of species (four species, including two legume and two non-legume in most of the works). Without testing the responses of yield to diversity within a larger species pool, the effect of the inclusion of other species remains unclear. Are there additional benefits to be gained by further increasing diversity? By exploring larger diversity, more interactions between- and within-functional groups (FG) could be investigated with the objective of disentangling the aspects of plant diversity promoting ecosystem functioning.

In addition, the joint assessment of the multiple services delivered by grasslands requires a holistic approach. Grasslands produce biomass fed to livestock, thus quality and stability of the production are important. Moreover grasslands are involved in nutrients and water cycling (Fig. 1.2), and thus need a wide panel of functions to be measured for an accurate assessment of their performance. The BEF relationship was characterized for multifunctionality through several works in natural ecosystems that used species richness as the metric for diversity (Zavaleta *et al.*, 2010; Byrnes *et al.*, 2014; Lefcheck *et al.*, 2015; Gamfeldt and Roger, 2017). However, the inclusion of other metrics of diversity (beyond richness) are crucial in intensively managed grasslands, despite the induced complications in multifunctionality assessment (Dooley *et al.*, 2015; Suter *et al.*, 2021). To explore the effect of plant diversity on grassland multifunctionality, research has to take their multiple purposes and challenges into account, and relate them to accurate metrics of diversity. Thus, assessments of the BEF relationship in intensively managed grasslands should lead to more precise recommendations about the characteristics of plant communities to establish (and their associated management), reflecting the multiple aspects involved in both grassland diversity and outcomes.

Considering the effect of a disturbance on the BEF relationship in intensively managed grassland

The effect of disturbance on the BEF relationship is crucial for adapting research results to a changing environment. Indeed, increased occurrence of extreme weather events is expected in near future (Orlowsky and Seneviratne, 2012; Shukla *et al.*, 2019). Vogel *et al.* (2012) showed that grassland resistance to drought events was reduced by management intensity, but increased by plant diversity. Thus, disturbances, such as weather extremes, should be particularly investigated under conditions of intensive grassland management, where their effect can be dramatic, taking the example of the 2018 fodder crisis in Ireland (DAFM, 2018; Falzoi *et al.*, 2019; Buras *et al.*, 2020).

Assessment of the resilience of intensive grasslands to a change in environment was often constrained to a restricted species pool and binary functional group interactions. Several aspects, such as resistance to drought, stability or weed invasion were separately proven to be enhanced by legume*non-legume interaction (Connolly *et al.*, 2018; Finn *et al.*, 2018; Haughey *et al.*, 2018). However, the principle of diversity associated resilience needs to be generalized to a larger range of species and functional groups, in intensively managed systems. Cong *et al.* (2014) introduced work on intensively managed agro-ecosystem without legume, but without assessing the effect of a disturbance. Further studies about the effects of a disturbance on the effect of plant diversity are necessary, as we know that environmental stress can induce changes in the BEF relationship under intensive management (Grant *et al.*, 2014). The inclusion of additional functional groups such as deep-rooted herbs with drought resistance traits could be explored as a way to stimulate the resilience of intensively managed grassland exposed to extreme weather events.

Which diversity for intensive agro-ecosystems?

To best promote ecological intensification, more research is needed for evidence-based recommendations for plant communities that optimize the BEF relationship. This section focuses on the precise determination of the facets of diversity to promote agro-ecosystem services. Thus, I explore some principles about how to best combine species and functional groups in a context of intensive management.

Choice of a species pool for intensive grasslands

In intensively managed grasslands, plant communities are designed and implemented at establishment, through sowing. Therefore, the species pool is not defined randomly from colonisation of surrounding species, but designed from plants with characteristics adapted to the intended management. Species will also be selected on their potential regarding the targeted ecosystem functions (e.g. yield). The ultimate selection narrows the pool to the single best species, often a grass monoculture, which is a common practice in temperate intensively managed grasslands. To explore further diversity, the pool can be enlarged with plants that fit the required characteristics, such as high yielding perennial species, adapted to grazing and regular fertiliser application. However, given that functional distance can enhance ecosystem functioning (Cadotte, 2013), species with distant traits represent a higher potential for stimulating BEF relationship, and thus grassland outcomes.

Metrics of diversity: the importance of species proportions to define a community

In intensively managed grasslands, the species pool being small enough, the contribution of each species or FG to a mixture outcome can be tracked. Consequently, rather than referring to the number of species in a community (e.g. species richness = 2 for a two-species mixture), it is possible to refer to the proportion of the component species or FG (e.g. a 70% grass, 30% legume mixture). Figures 1.3 and 1.4 highlights that when species deliver contrasting performances for a given function, the outcome of a mixture would vary depending on the proportion of each species. In a two-species example such as sp1 and sp3 (represented by the axis connecting these two in Fig 1.3), a mixture of 80% of sp1 and 20% of sp3 would deliver 4.2 units, against 7.8 units for a mixture of 20% of sp1 and 80% of sp3.

When investigating mixtures, interactions between species are to be considered, as they may have great impact on the mixtures' outcomes (Isbell *et al.*, 2009; Kirwan *et al.*, 2009; Justes *et al.*, 2021). Plant interactions may depend on the relative abundance of the species involved in the interaction (Wilsey and Polley, 2004; Polley *et al.*, 2007; Brophy *et al.*, 2017b). In species-rich grasslands, the amount of possible interactions limits their assessment. Brophy *et al.* (2017a) suggest to group them by functional units prior to analysis of functional group contribution and interactions through a method adapted to lower identities (Kirwan *et al.*, 2009). In intensively managed grasslands, linking interaction effects to species composition and proportions is eased by the typically more restricted species pool of two to ten species. One way to further simplify the link between plant interactions and ecosystem function is summarizing interaction into the single term of evenness (Kirwan *et al.*, 2007). However, this approach assumes that all species interact at the same magnitude (Fig. 1.3,b). This assumption may not be reasonable for all communities, as Nyfeler *et al.* (2009) showed that grass-legume interactions were stronger than within-FG ones. The characterisation of specific interactions requires then to quantify the effect of each species or FG pairwise interaction in a community (see Fig. 1.3,c for an example). Keeping in mind that proportions are continuous (between 0 and 1), contrarily to species richness, the assessment of the BEF relationship from composition and relative abundance requires experimental and analytical adjustments.

Experimental assessment of the biodiversity – ecosystem functioning relationship under intensive management

After identifying plant diversity as a potential positive management factor for agricultural systems, I identify outstanding knowledge gaps and suggest experimental approaches to disentangle them. I introduce the simplex experimental design, and why its features are suitable to research on mixtures. I then discuss an appropriate experimental design with which to test the effect of abiotic factors on mixture communities. Finally, I attempt to disentangle the assessment of the links between the complex structure of diversity and the multiple outcomes of agro-ecosystems.

Knowledge gaps in BEF relationship for agro-ecosystems

The first limitation in investigating BEF relationship is the definition of diversity. As introduced (above), measures of diversity vary a lot, with separate studies using either richness, evenness, taxonomic versus functional diversity, etc.

Experiments in intensive agro-ecosystems often treat plant communities' composition as a categorical variable, comparing monoculture and mixtures through ANOVAs, without investigating the effect of varying species proportions (Sanderson *et al.*, 2004). Over the past two decades, some research works intended to refine recommendations to the species proportion level for accurate implementation on farms. Figure 1.3 illustrate the importance of considering species relative abundance, as the balanced mixtures (e.g. two-species mixture highlighted in blue) can differ strongly to other communities of same composition but varying proportions (e.g. corresponding segments). In addition, experimentations that simulate farming practices are necessary to ensure transferability of results to real agro-ecosystems. Consistent research had been performed on natural ecosystems, but the works investigating BEF relationship under intensive agricultural management are less common.

Beyond the complexity of plant diversity already introduced, there is a need for greater understanding of the mechanisms that drive the BEF relationship across multiple ecosystem functions. Indeed, yield remains the central focus of intensive agro-ecosystems, but several other agro-environmental functions are important to consider, such as nutrient use efficiency, quality of the production, resistance to weed invasion, etc.

Finally, a critical aspect to investigate is how the BEF relationship maintains under a range of abiotic factors. Indeed, the effects of climate change threatens agro-ecosystems (Hopkins and Del Prado, 2007; Coronese *et al.*, 2019; Hossain and Li, 2020; Carozzi *et al.*, 2021). Most concerns seem concentrated about fluctuations in water supply, with drought in the foreground (Fry *et al.*, 2014; Felton *et al.*, 2021; Hahn *et al.*, 2021). Any aspect of the BEF relationship is suspected to change in case of variations in abiotic factors (Grant *et al.*, 2014). To promote the accuracy of results in a changing environment, it is crucial to cross the assessment of BEF relationship with the potential occurrence of a weather disturbance, in line with projections for future conditions.

Experimental design to investigate BEF relationship

a. Diversity-Interactions modelling approach and implications for experimental design

The main challenge when investigating the effect on a response from mixing separate components is the infinity of possible combinations in the mixture. Experimental design requires sufficient representativeness of any combination to allow a regression across all possible mixtures.

Simplex designs allow to extrapolate responses across all combinations and components from a given pool (Cornell, 2011). To enable extrapolation, this design requires a good representation of communities distributed along the edges of the response shape and an even spread of the combinations across the interior of the design space. To illustrate this, Figure 1.3 shows an example of a simplex design for a mixture experiment using a three-species pool. The communities are highlighted by the coloured points, showing their even spread.

An effective representation of the vertices of the design space informs about each species contribution to the outcome. In the case where components of the mixture are expected to interact with each other, as is the case in grasslands (Gibson *et al.*, 1999), replicates of the communities on the edges of the simplex are also required to ensure proper determination on pairwise interactions (e.g. two-species mixtures highlighted in blue in Fig 1.3). Communities in the inner space of the design refine knowledge of the effect of species contributions and interactions, as well as informing whether higher-order terms are needed (e.g. three-species mixtures highlighted in purple and red in Fig 1.3). Cornell (2011) assumes the minimum number of mixtures required in the design to be the number of species in the pool plus one. The number of replicates for each mixture is determined by the objectives of the study (with focus on evenness, binary interactions, dominance effects, etc.). A wide and regular spread of the mixtures across the design would avoid leverage effect of isolated observations. Replication of reference points such as centroid (the equi-proportional mixture of all species from the pool, red point in Fig. 1.3) improves the confidence of prediction in areas of interest in the design (see Fig. S1.1 for pictures of a monoculture and a mixture).

The simplex is an optimal design to provide data for modelling the contribution of species and their interactions in the delivery of outcomes. This approach developed by Kirwan *et al.* (2009), was called "Diversity-Interactions modelling". As stated in its name, the modelling method enables characterising the contribution of each species to a mixture (species identity effects), as well as the additional effect of interactions between species (interaction effects). The Diversity-Interactions approach applied to a three-species simplex would be modelled as follows:

$$y = \beta_1 P_1 + \beta_2 P_2 + \beta_3 P_3 + \delta_{12} P_1 P_2 + \delta_{13} P_1 P_3 + \delta_{23} P_2 P_3 + \epsilon$$

Where y is the modelled outcome, β_i are the identity effects, δ_i are the interaction effects and P_i are the proportions for each species. If $P_1 = 1$, P_2 and $P_3 = 0$, then $E[y] = \beta_1 P_1 = \beta_1$. If $P_1 = 0.5$, $P_2 = 0.5$, and $P_3 = 0$, then $E[y] = \beta_1 P_1 + \beta_2 P_2 + \delta_{12} P_1 P_2 = \beta_1 0.5 + \beta_2 0.5 + \delta_{12} 0.5 \times 0.5$. You cannot change P_1, while keeping P_2 and P_3 constant; however, predicting from the Diversity-Interactions model is achieved in the same way as any other multiple regression model: by plugging in the values of the covariates (which are proportions and must sum to one). As with any multiple regression model, you can predict from the model for any set of predictor values that are within the range of the data, even if the exact set of values are not included in the dataset. This method has the distinct advantage (over ANOVA-based approaches for BEF studies) of allowing estimation of the performance of a wide range of communities that were not included in the field experiment but are encompassed by the design space.

b. Measuring the impact of an abiotic factor

Investigating how the BEF relationship would react to a disturbance in growing environment is of great interest to prepare agro-ecosystems that are facing future climatic challenges. Climate change threatens agro-ecosystems with fluctuations in precipitation and temperature (Rosenzweig *et al.*, 2001; Stocker *et al.*, 2013; Emadodin *et al.*, 2021). In parallel, mineral fertiliser use is criticised for its embedded environmental impact (Leip *et al.*, 2015; Shukla *et al.*, 2019). Taking these abiotic factors into account when assessing grassland performances can help to inform the viability of agro-ecosystems in the face of changes in environmental conditions.

To investigate the effect of abiotic factors like variations in rainfall or fertility, experimental work is required with contrasted levels of each factor. A multi-site experiment allows to benefit from natural variations of rainfall or fertility, but is logistically challenging to implement and analyse as multiple other site-to-site factors can affect results. The other method consists of a within-site manipulation of the factors of interest. This eases the control of other factors as they can be considered uniform across the experiment if the design respects key aspects (random allocation of treatments, within-site uniformity).

For the present thesis, I varied water supply to an extreme degree (drought), to test the effect of such a disturbance on the BEF relationship. Summer drought was selected as the disturbance, due to climate change predictions and their associated threats to intensively managed agro-ecosystems (Hopkins and Del Prado, 2007; Carozzi *et al.*, 2021). The assessment of a weather disturbance effect is generally enabled by applying the disturbance as a treatment and compare it to an undisturbed control. In field experiments, the spatial variability of a field cannot be corrected and has to be taken into account for the design. However the designs for Diversity-Interactions modelling generally involve a large number of plots and uneven number of replicates across mixtures. To minimise the effect of spatial variation, a split-plot design was preferred as it enables to keep control and drought side-by-side, reducing external factors of variance in the field (Kowalski *et al.*, 2002). Moreover, split-plot designs are compatible with the simplex design, and thus can be analysed by a Diversity-Interactions model, counting within-plot gradient as an extra treatment. This method also eased the logistical aspects of drought simulation by enabling implementation of plot-scale devices to manipulate water supply (see Fig. S1.2, S1.3 and S2.1).

Soil fertility is artificially promoted through input of mineral fertiliser in intensively managed agro-ecosystems. In anticipation of the intended reduction in fertiliser use from intensive European agro-ecosystems (European Commission, 2020), we adjusted fertiliser inputs in our experiment to match nitrogen deposition from grazing ruminants taking local average stocking rate (approx. 2 livestock units ha^{-1}), with no extra off-farm inputs (150 kg N ha^{-1} yr^{-1}). However, this rate does not reflect practices of the most intensive systems where extra mineral fertiliser is applied. Thus we included replicates of a grass monoculture receiving 300 kg N ha^{-1} yr^{-1} as a low-diversity, high-input comparison in order to match local conventional practices (150 kg N ha^{-1} yr^{-1} of animal deposition plus 150 kg N ha^{-1} yr^{-1} of mineral inputs). Fertiliser rates values were inspired from the lower range of practices reported by Dillon *et al.* (2020). The rainfed and drought treatments were also applied to the extra plots of low-diversity, high-input comparison, to assess the response of fertiliser increase to such weather disturbance.

PhD objectives and overview of thesis

The objective of the present work is to refine knowledge about the BEF relationship by linking detailed description of plant diversity to multiple functions under two contrasting growing conditions. Through experimentation, and keeping in mind transferability of the results to real farms, I investigated the following research aims:

- Investigate larger diversity and the effect of within and between FG interactions, in intensively managed grassland,
- Quantify the effects of abiotic factors such as weather disturbance on BEF relationship under intensive management,
- Compare the effects of increased diversity to a conventional farming approach (based on higher fertiliser input),
- Assess the BEF relationship across a larger panel of ecosystem functions.

In this work, a total of six species from three functional groups (FG) were selected, in order to explore how diversity of grassland species or FG would drive the delivery of ecosystem functions. The communities built from this six-species pool would thus comprise between one and six species and between one and three FGs. This is quite novel compared to previous research in intensively managed systems, which generally (but not always) comprise a maximum of four species. Thus, in addition to the well-investigated grass and legume FG, the inclusion of herb species is studied. This design and size of the species pool enables simultaneous quantification of within- and between-FG interactions which is also highly novel. All communities were crossed with two levels of water supply (rainfed control and nine-week experimental drought), introducing the effect of a severe weather event.

The Diversity-Interactions approach allows investigation of the BEF relationship through a detailed description of the complex structure of diversity. The effect of different aspects of diversity can be crossed or added to a treatment effect to explain associated variance in the delivery of a response. Benefitting from this approach, the Chapter 2 of this thesis investigates the effect of species identities, FG interactions and drought on grassland annual yield under intensive management. Thus, Chapter 2 disentangles which aspects of plant diversity enhances the BEF relationship, and how this is affected by drought. The effect of diversity on annual yield is also compared to the effect of increased fertiliser yield in order to relate ecological intensification to intensification from higher use of inputs.

Chapter 3 investigates the legacy effect of plant diversity and drought by measuring the residual effect of the preceding diversity*drought experiment on the following crop in a crop rotation (Fig. S1.4). Thus, Chapters 2 and 3 link the complex structure of plant diversity to single ecosystem functions, under contrasted weather conditions (occurrence of summer drought or not). They test the application of plant species identities and interaction effects within intensively managed farming system as a practical tool to enhance ecological intensification.

Chapter 4 links the effect of diversity and abiotic factors to multiple ecosystem functions. Using a multivariate analysis to quantify multifunctionality in the agronomic plant communities, I deepen the exploration of the BEF relationship by investigating the relationships between diversity and multiple interdependent ecosystem functions, using a statistical approach that facilitates direct comparisons. The effect of drought on these relationships is also studied.

This work addresses several knowledge gaps regarding the effect of diversity and abiotic factors on the delivery of ecosystem functions. In Chapter 5, I relate my conclusions to the literature, identify limitations and suggest orientations for future work.

Supplementary material

Figure S1.1. Picture of two plots with contrasting diversity, from the experimental field presented in this study. The grassland community on the left is a *L. perenne* monoculture and the one on the right is an equi-proportional six-species mixture (centroid).

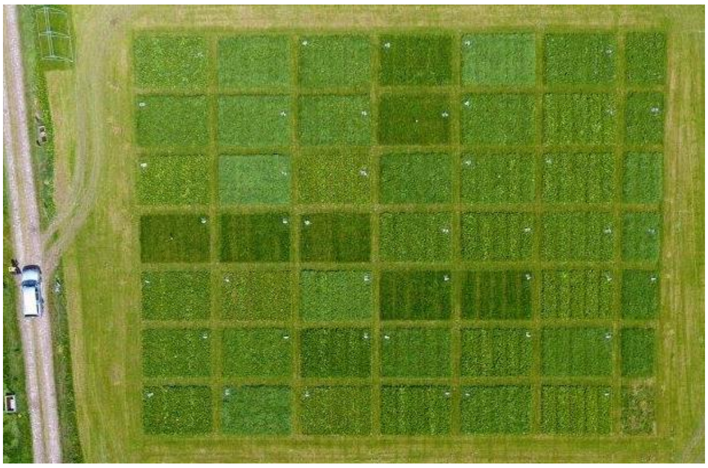


Figure S1.2. Drone picture of the experimental field in May 2018. Different shades of green can be spotted due to differences in plant composition.

Figure S1.3. Drone picture of the experimental field in July 2018, with rainout shelters applied to one half of each plot. See Fig. S2.1 for a closer picture of a rainout shelter.

Figure S1.4. Drone picture of the experiment, after each plot was reseeded with a *L. multiflorum* monoculture (July 2020). Picture was taken during a harvest, performed with a Haldrup harvester.

Chapter 2

The work presented in this chapter forms the basis for the journal paper Grange et al 2021 (Journal of Applied Ecology 58, 1864-1875). The work was carried out in collaboration with the listed co-authors; I implemented the experiment, and I led the statistical modelling work, preparation of results and writing of the paper.

Plant diversity enhanced yield and mitigated drought impacts in intensively managed grassland communities

Guylain Grange, John A. Finn, Caroline Brophy

Abstract

There is a global requirement to improve the environmental sustainability of intensively managed grassland monocultures that rely on high rates of nitrogen fertiliser, which is associated with negative environmental impacts. Multi-species grass-legume mixtures are a promising tool for stimulating both productivity and sustainability in intensively managed grasslands, but questions remain about the benefit of increasing the diversity of plant functional groups.

We established a plot-scale experiment that manipulated the diversity of plant communities from a six-species pool comprising three functional groups: grasses, legumes and herbs (two species each). Communities were grown as monocultures, or mixtures of two to six species, following a simplex design. This allowed us to quantify species' identity effects and functional group interaction effects. To investigate the impact of severe weather events, main plots were split and two levels of water supply, 'rainfed' and a two-month experimental drought, were applied. Mineral nitrogen fertiliser was applied at 150 kg ha^{-1} yr^{-1}, and a *Lolium perenne* monoculture received 300 kg ha^{-1} yr^{-1} (300N) as a highly fertilised comparison. Annual aboveground biomass was measured for two years. The data and code are publicly available (https://doi.org/10.5061/dryad.qfttdz0g9)

We found a strong positive effect on yield due to functional group interactions. Multi-species communities with three functional groups yielded more than the best-performing monoculture over a large range of different relative abundances of the three functional groups. The highest diversity community outperformed the 300N *L. perenne* under rainfed conditions.

Although species' monoculture yields were generally reduced by the experimental drought, the interaction effects were not affected by drought and remained sufficiently large for the six-species mixture under drought to have similar yields to both the best-performing monoculture and the 300N *L. perenne* under rainfed conditions.

Synthesis and applications. Plant diversity can result in higher yields, mitigate the yield risks associated with extreme weather events, and displace fertiliser inputs and their embedded greenhouse gas emissions. Multi-species swards offer a practical, farm-scale management action to enhance the sustainability of intensive grassland production.

Key-words: disturbance, drought, ecological intensification, mixtures, transgressive overyielding, yield

Introduction

Intensively managed grassland systems underpin food production and agricultural economic activity in areas with a temperate climate. Many of these systems rely on grass monocultures that receive high inputs of fertiliser for high productivity of meat and milk products. Globally, livestock production remains responsible for a substantial proportion of anthropogenic greenhouse gases emissions (Uwizeye *et al.*, 2020); one mitigation action is to reduce reliance on large amounts of inorganic nitrogen (N) fertiliser, which has multiple negative environmental impacts (Peeters, 2009). In addition, grasslands rely on regular water supply, while the risk of severe weather events increases due to climate change (Falzoi *et al.*, 2019). There is an urgent need to improve the environmental performance of grassland systems and improve their resilience to shocks such as fluctuations in precipitation and fertiliser cost (Rosenzweig *et al.*, 2001). Compared to more extensive grasslands, drought events can have harsher economic impacts on intensively managed grasslands (Finger *et al.*, 2013).

Multi-species grassland mixtures have been proposed to mitigate environmental impacts associated with intensively managed grassland monocultures, while maintaining productivity (Trenbath, 1974; Luscher *et al.*, 2014). This is achieved through multiple synergistic interactions among species, including complementarity in resource use (e.g. acquisition of soil nutrients and water; symbiotic N_2 fixation, and utilisation of light). Multi-species mixtures in intensively managed systems have been shown to achieve not just overyielding (Sanderson *et al.*, 2013; Moloney *et al.*, 2020), but also transgressive overyielding (when a mixture performance exceeds that of the best-performing monoculture) (Trenbath, 1974). The legume proportion was a key determinant of transgressive overyielding in grass-legume mixtures, where four-species mixtures with low nitrogen and legume composition of ~30-50% out yielded 100% grass swards with high nitrogen application (Nyfeler *et al.*, 2009). Finn *et al.* (2013) found a regular occurrence of transgressive overyielding in a three-year common experiment with four-species grass-legume mixtures performed across 31 international sites.

However, most manipulation experiments with intensively managed multi-species grasslands have been constrained to between two and four species, with a rare inclusion of herbs (Sanderson *et al.*, 2004). Results are often analysed through ANOVA-based approaches, which limits any extrapolation of the conclusions beyond the composition and evenness of the specific communities that were empirically tested. Relatively few experimental studies have investigated the degree to which plant diversity in intensively managed grassland systems can confer resilience to environmental shocks. Haughey *et al.* (2018) and Finn *et al.* (2018) related species richness (1, 2 and 4-species mixtures from a species pool of one grass, two legumes and one herb) to higher yield, greater drought resistance and higher yield stability. Komainda *et al.* (2020) found that drought tolerance was related to species identity and functional group diversity. We further develop this topic by increasing the number of species to six in a field experiment that aligns with farmed conditions, and using a design that allows us to quantify both within- and between-functional group interactions.

If multi-species swards can maintain (or increase) yield with lower inputs, improve environmental performance, and help resist disturbances, compared to monocultures, they can play a key role in the sustainable intensification of intensively managed grasslands. Here, we use assembled communities in a six-species system (two grass, two legume and two herb species) to investigate the separate effects of species identities and their interspecific interactions on total annual yield. We also manipulated water supply (two levels) by imposing an experimental drought that was compared with the rainfed control. A high level of nitrogen application (300 kg ha^{-1} yr^{-1} of N) on *L. perenne* (300N *L. perenne*) was included for comparison with all other communities that received 150 kg ha^{-1} yr^{-1} (150N).

We used Diversity-Interactions modelling (Kirwan et al. 2009) to model ecosystem function (the response, total annual yield, in this case). This is a multiple regression approach with predictors being the species proportions (species identity effects) and their interactions (see Fig. 2.1 for an example for a two-species mixture). The water supply treatment factor and interactions including it are also tested in the multiple regression model. Replication in our study is across the set of continuous species proportions (in a simplex design) and Diversity-Interactions modelling is a type of response surface analysis, which is well established statistical theory for this type of data (see Cochran and Cox, 1957).

The specific aims of this two-year field experiment were to:

1) Quantify the effects of plant diversity through analysis of species' identities and their interactions on total annual yield and explore whether species' interactions were driven by functional group membership.
2) Investigate the ability of mixtures to mitigate drought impact on total annual yield.
3) Investigate whether and to what extent transgressive overyielding occurred.
4) Compare total annual yield with that of the 300N *L. perenne* community (under rainfed and experimental drought conditions).

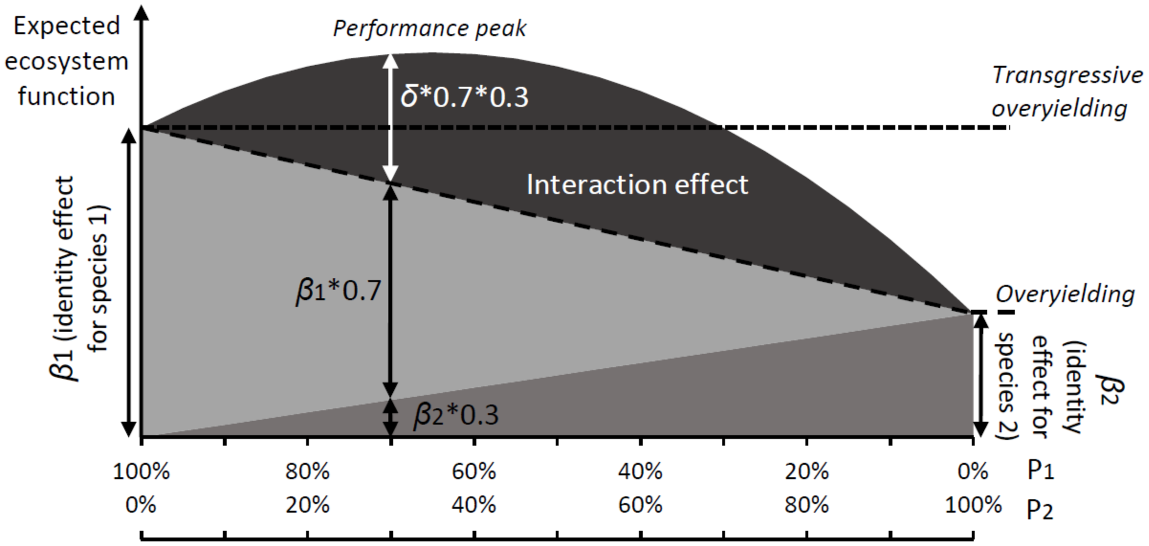


Figure 2.1. Illustration of the Diversity-Interactions modelling approach of an ecosystem function (*y*, here total annual biomass production) in a two-species system. For the equation $y = \beta_1 P_1 + \beta_2 P_2 + \delta P_1 P_2 + \varepsilon$ (adapted from eqn. 2.1 for two species), we illustrate the expected ecosystem function for all possible communities involving the two species, ranging from a monoculture of species 1 (on the left), to a monoculture of species 2 (on the right), and all possible two-species mixtures in between (e.g., 50:50% in the centre point). The identity effect for species 1 is β_1 and this is the expected performance of species 1 in monoculture (similarly it is β_2 for species 2). In mixture, the expected performance is a weighted average of the identity effects ($\beta_1 P_1 + \beta_2 P_2$) plus the interaction effect scaled by the product of the two proportions ($\delta P_1 P_2$). As highlighted below, the expected ecosystem function for the 70:30% mixture is β_1*0.7 + β_2*0.3 + δ*0.7*0.3. In this example, species 1 performs better than species 2, and so has a higher identity effect; and δ is positive reflecting synergistic interaction, however, it could also be negative or 0. These concepts scale up for systems with >2 species. Because the ecosystem function of interest is the biomass production in this case, the sloped dashed line shows the 'overyielding' threshold, i.e., where a mixture outperforms the weighted monoculture performance. The horizontal dashed line shows the 'transgressive overyielding' threshold, i.e., where a mixture outperforms the best performing monoculture.

Materials and Methods

Site and design

A plot-scale experiment was established in April 2017 at Johnstown Castle, Wexford, in the south-east of Ireland (52°17'57.8"N, 6°30'23.3"W, 71 m a.s.l.). A previously grass-dominated field (with sandy-loam soil at pH 5.7, and total carbon and nitrogen content of 12.2 and 2.45 g kg^{-1} of soil respectively), was ploughed and reseeded with a total of 43 plots, each of area 35 m^2 (5 m x 7 m). Replicates of 19 different forage plant communities were randomly assigned to plots, combining six different species. The species were chosen from perennial forage species adapted to most Irish soils, climate and intensive management, with high yield potential, and to represent three contrasting functional groups (FGs): grasses (*Lolium perenne, Phleum pratense*), legumes (*Trifolium pratense, Trifolium repens*) and herbs (*Cichorium intybus, Plantago lanceolata*). The sown communities comprised six monocultures and 13 mixtures, from two to six species. Following a simplex design (Cornell, 2011), species proportions were 100% of the advised seeding weight for monocultures; 50% of each species from the same FG for the two-species mixtures; 25% of each species from two FGs for the four-species mixtures, and; 16.7% of each species for the six-species mixtures centroid. The five-species mixtures were dominated by each species in turn, such that there was 60% of one species and 10% of the four species from the other two FGs. Due to a mistake in species proportions at establishment, one plot was removed from the design. Replicates for the 19 unique plant communities are given in Table S2.1.

Communities received 150 kg ha^{-1} of nitrogen fertiliser per annum (150N), split into five applications. Four replicates of a *Lolium perenne* monoculture receiving 300 kg ha^{-1} of nitrogen were included as an extra community for a high fertiliser comparison (300N). Background fertilisation of 60 kg ha^{-1} of phosphorus and 300 kg ha^{-1} of potassium was split in three applications, to ensure these nutrients were not limiting. Two water supply treatments were applied, using a split plot design. Each main plot was divided into two 5 m x 3.5 m split plots. One half (randomly chosen) received natural water supply over the year ('rainfed'), while a two-month summer drought was simulated on the other half, using rainout shelters ('drought'). Beginning the year after establishment, measurements were recorded on each sub plot for two full growing seasons (i.e., from March 2018 to October 2019).

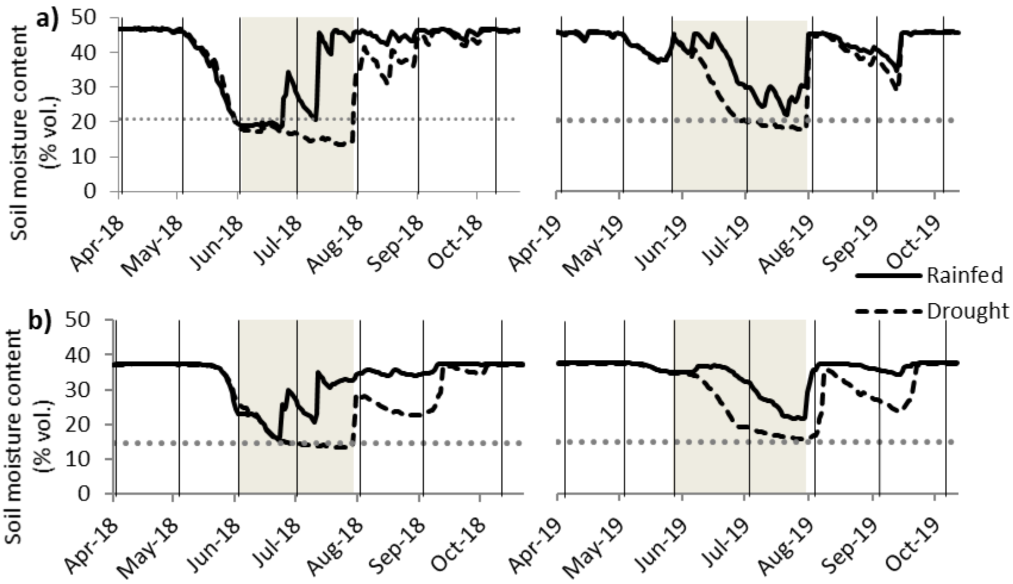


Figure 2.2. Volumetric soil moisture content (%) at 10cm (a) and 40cm (b) depth of rainfed and drought sub plots of three centroid and two *Lolium perenne* monoculture plots. The values displayed are the average of the five plots (smoothed by week). Grey dashed line shows the moisture content corresponding to a soil matric potential of -1.5MPa. It is considered that plant water absorption is obliterated below this threshold (permanent wilting point). Vertical lines represent harvest dates.

Drought implementation and soil moisture monitoring

The rationale for the drought duration was based on the objective of inducing a significant impact on plant growth. This decision was supported by the occurrence of two such drought events of similar duration in the past ten years (2013 and 2018). Moreover, climate models predict a 12 to 40% increase in summer dry periods by 2050 (Nolan et al, 2017). Rainout shelters were constructed using metallic frames covered with transparent plastic foil (SunMaster SuperThermic, 150 µm, XL Horticulture, UK). These tunnel-shaped shelters were open on both extremities and 0.3 m width apertures on the top and sides helped ventilation (photograph provided in Fig. S2.1). Air temperature and humidity were recorded using Tynitag© TGP-4500 monitors. No significant difference was measured in temperature nor humidity in and out of the shelters. To monitor soil water availability, soil water potential probes were installed at 10 cm and 40 cm belowground on both sub plots of five randomly chosen plots within the replicates of *L. perenne* monocultures and six-species mixtures. Water potential was measured and converted to volumetric soil moisture content, using HYPROP equipment (METER group (UMS, 2015)), fitted with Fredlund-Xing model (Fig. 2.2). Due to exceptionally low rainfalls in summer 2018, the rainfed control of each plot was irrigated to ensure a different water supply between the two treatments. A total of 90 mm was supplied to these sub plots over three watering events (28/06/2018, 04/07/2018 and 18/07/2018). Information about water supply can be found in Table S2.2. In order to assess the effect of rainout shelters on light availability, photosynthetically active radiations (PAR) were measured inside and outside each shelters, twice during the drought application period. The measurement were performed above the canopy using a ceptometer (AccuPAR LP80, Decagon Devices, USA).

Data collection and analysis

All plots were harvested by cutting at 4.5 cm height on seven occasions over each year in 2018 and 2019 with a Haldrup F-55 harvester (harvest dates in Fig. 2.2). From a central 1.5m strip of each plot, fresh biomass was weighed by the harvester, and dry matter content determined by oven drying a representative sub-sample of approximately 100 g. Dry matter yield was summed for each year to obtain total annual aboveground biomass production, and was analysed using Diversity-Interactions modelling (Kirwan *et al.*, 2009; Connolly *et al.*, 2013; Brophy *et al.*, 2017a). A Diversity-Interactions model is a multiple regression model, with response y (here dry matter yield), and continuous predictors that are proportions and their interactions (here sown proportions are used), as illustrated in Fig. 2.1 for two species. The unique aspect of Diversity-Interactions models, compared to other multiple regression models, is that the species proportions for any community must sum to one. Typically, a multiple regression model coefficient, say β_1 for predictor X_1, is interpreted in the context of keeping all other X predictors constant. But when the predictors are proportions summing to one, it is not possible to change one proportion while keeping others constant.

Here, we present results from the model fitted to our data based on the six individual species identity effects, the pairwise interactions within each of the three functional groups, and the pairwise interactions across the functional groups (eqn. 2.1). A mixed model was used to account for the two sources of variation due to the split-plot design (main-plot and sub-plot variation). In addition, an 'unstructured' error structure was assumed (after comparison to suitable alternatives) for the repeated measures on sub plots over the two years. The following linear mixed model was fitted to the observed experimental data:

$$\begin{aligned} \text{DMY} = {} & \textit{Year} * \textit{Treatment} * [\beta_1 P_{\text{Lp}} + \beta_2 P_{\text{Pp}} + \beta_3 P_{\text{Tp}} + \beta_4 P_{\text{Tr}} + \beta_5 P_{\text{Ci}} + \beta_6 P_{\text{Pl}} \\ & + \delta_{\text{GG}} P_{\text{Lp}} P_{\text{Pp}} + \delta_{\text{LL}} P_{\text{Tp}} P_{\text{Tr}} + \delta_{\text{HH}} P_{\text{Ci}} P_{\text{Pl}} \\ & + \delta_{\text{GL}}(P_{\text{Lp}} + P_{\text{Pp}})(P_{\text{Tp}} + P_{\text{Tr}}) + \delta_{\text{GH}}(P_{\text{Lp}} + P_{\text{Pp}})(P_{\text{Ci}} + P_{\text{Pl}}) + \delta_{\text{LH}}(P_{\text{Tp}} + P_{\text{Tr}})(P_{\text{Ci}} + P_{\text{Pl}}) \\ & + \gamma X_{\text{N}}] + \varepsilon \end{aligned} \qquad \text{Eqn. 2.1}$$

Where DMY was the annual dry matter yield, P was the sown proportion of a species (with subscripts Lp = *L. perenne*, Pp = *P. pratense*, Tp = *T. pratense*, Tr = *T. repens*, Ci = *C. intybus,* Pl = *P. lanceolata*) for plots fertilised under the 150N regime. Each β_i term stands for a species identity effect (explained for a two-species system in Fig. 2.1), i.e., the expected annual dry matter yield for a monoculture of species *i* under 150N fertiliser ($P_i = 1$, $P_{\neq i} = 0$) is β_i, and when $P_i < 1$, the species identity contribution to the mixture is $P_i\beta_i$. The extra factor X_N was coded 0 and 1 for subplots that received 150N and 300N fertiliser, respectively. There were only eight subplots (four rainfed and four drought) that received the 300N fertiliser, and each were monocultures of *L. perenne*. The covariate P_{LP} was coded 0 for these high N subplots, therefore is the expected average yield of 300N *L. perenne* monocultures. We restrict our inference related to this high N treatment, i.e., we do not try to estimate how species other than *L. perenne* would respond to the high N treatment.

Contributions to yield of pairwise interactions within and between functional groups (G = grass, L = legume, H = herb) were estimated by the six δ terms, scaled by sown proportions. For example, in a six-species equi-proportional mixture (centroid), each species had a sown proportion of 1/6; in this case 'within' FG interactions (δ_{GG} or δ_{LL} or δ_{HH}) are multiplied by the product of species proportions from these FGs $P_{Lp}P_{Pp}$ or $P_{Tp}P_{Tr}$ or $P_{Ci}P_{Pl} = (1/6)^2 = 0.02778$; while 'between' FG interaction terms, (δ_{GL}, δ_{GH}, δ_{LH}) are multiplied by $(1/6 + 1/6)(1/6 + 1/6) = 0.3333^2 = 0.1111$. All terms in the model were crossed with the water supply treatment factor (rainfed and drought) and the year factor (2018 and 2019). The model in eqn. (2.1) and variants of it were compared using AICc (Burnham and Anderson, 2002). Models were fitted using 'proc mixed' in SAS (SAS Institute, 2013) using either maximum likelihood (for comparisons of fixed effects) or restricted maximum likelihood (for comparisons of error structures and final model presentation).

Results

Climatic conditions were very different between 2018 and 2019. An exceptionally dry summer in 2018 contrasted with heavy rain in early summer of 2019. Soil moisture patterns differed, and the drought induced by the shelters varied from extreme at both soil depths in 2018 to severe for shallow and limited for deeper soil in 2019 (Fig. 2.2). The total amount of rainfall removed by the rainout shelters was 122 and 98 mm for 2018 and 2019 respectively. Also, the effect of shelters on light was a reduction of 25% of PAR.

Selection of Diversity-Interactions model

The final Diversity-Interactions model selected was:

$$
\begin{aligned}
\text{DMY} = {} & \mathit{Year} * \mathit{Treatment} * [\beta_1 P_{Lp} + \beta_2 P_{Pp} + \beta_3 P_{Tp} + \beta_4 P_{Tr} + \beta_5 P_{Ci} + \beta_6 P_{Pl} \\
& + \mathit{Year} * [\delta_{GG} P_{Lp} P_{Pp} + \delta_{LL} P_{Tp} P_{Tr} + \delta_{HH} P_{Ci} P_{Pl} \\
& + \delta_{GL}(P_{Lp} + P_{Pp})(P_{Tp} + P_{Tr}) + \delta_{GH}(P_{Lp} + P_{Pp})(P_{Ci} + P_{Pl}) + \delta_{LH}(P_{Tp} + P_{Tr})(P_{Ci} + P_{Pl})] \\
& + \mathit{Year} * \mathit{Treatment} * [\gamma X_N] + \varepsilon
\end{aligned}
$$

Eqn. 2.2

Eqn. (2.2) varies from eqn. (2.1) only in that the interaction terms (δ_{GG}, δ_{LL}, δ_{HH}, δ_{GL}, δ_{GH} *and* δ_{LH}) were not crossed with the water supply treatment. The AICc was reduced by 40.5 for the fitted model in eqn. (2.2) compared to eqn. (2.1). Alternative assumptions about the interactions did not improve the model AICc; this included testing for non-linearity in the interactions (as described by Connolly *et al.* (2013)) and testing for three-way interactions. The final model parameter estimates are shown in Table 2.1a; the remainder of the results section will interpret this fitted empirical model. To illustrate with an example: if we predict for a two-species 50:50 mixture of the two legumes for rainfed conditions under regular N conditions in 2018 using the values in Table 2.1 we get: $\widehat{DMY} = 11.7 * 0.5 + 10.3 * 0.5 + 2.87 * 0.5 * 0.5 = 11.72$. All other species proportions and X_N are 0, so all other coefficients in the model are multiplied by 0 and do not feed into this mixture prediction (this prediction is done similarly to prediction from any multiple regression model, as described in the Methods section).

Table 2.1. (Next page). Parameter estimates (a) and predictions from selected communities (b) from the mixed model shown in eqn. 2.2; the units are t ha^{-1} yr^{-1}. The standard errors for the two-year average values take year to year co-variance into account. The *Diff* columns give the level of significance of the difference between the rainfed and drought treatments (*** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, † P ≤ 0.1, ns: not significant). In part (a), FG interaction estimates in bold indicate significant parameters (P ≤ 0.05). In part (b), 'Monoculture average' is the mean of all 150N species identity effects. 'Six-species centroid' and 'Four-species' are predictions from equi-proportional mixtures, including both species from each FG. 'Five-species' are three FG mixtures where one species was dropped (0% for the named species, and 20% for each of the five others). For the two-year average (only), hash signs ($^{\#}$) denote the transgressive overyielding, i.e., communities significantly outperforming the highest yielding monoculture (*P. pratense*) (α = 0.05).

		2018			2019			two-years average		
a) Modelled fixed effects		Rainfed	Drought	*Diff*	Rainfed	Drought	*Diff*	Rainfed	Drought	*Diff*
Species identity (β)	*Lolium perenne*	9.2 *±0.44*	8.3 *±0.44*	*	9.1 *±0.35*	8.6 *±0.35*	*†*	9.2 *±0.29*	8.4 *±0.29*	*
	Phleum pratense	11.1 *±0.46*	10.1 *±0.46*	*	10.2 *±0.37*	9.2 *±0.37*	**	10.7 *±0.30*	9.6 *±0.30*	**
	Trifolium pratense	11.7 *±0.44*	8.9 *±0.44*	***	8.3 *±0.35*	7.2 *±0.35*	***	10.0 *±0.29*	8.0 *±0.29*	***
	Trifolium repens	10.3 *±0.44*	8.4 *±0.44*	***	9.7 *±0.35*	8.8 *±0.35*	*	10.0 *±0.29*	8.6 *±0.29*	***
	Cichorium intybus	8.6 *±0.44*	8.5 *±0.44*	*ns*	8.5 *±0.35*	7.5 *±0.35*	**	8.5 *±0.29*	8.0 *±0.29*	*†*
	Plantago lanceolata	10.6 *±0.44*	8.8 *±0.44*	***	10.5 *±0.35*	10.2 *±0.35*	*ns*	10.6 *±0.29*	9.5 *±0.29*	**
FG interaction (δ)	Grass * Grass	0.5 *±2.13*			2.2 *±1.81*			1.4 *±1.44*		
	Legume * Legume	2.9 *±2.10*			2.9 *±1.79*			**2.9** ***±1.43***		
	Herb * Herb	-3.0 *±2.10*			1.0 *±1.79*			-1.0 *±1.43*		
	Grass * Legume	**6.1** ***±1.75***			**6.7** ***±1.49***			**6.4** ***±1.18***		
	Grass * Herb	**4.8** ***±1.75***			2.6 *±1.49*			**3.7** ***±1.18***		
	Legume * Herb	**5.8** ***±1.72***			**8.9** ***±1.47***			**7.3** ***±1.17***		
300N *Lolium perenne* (γ)		10.7 *±0.41*	10.4 *±0.41*	*ns*	10.3 *±0.32*	10.4 *±0.32*	*ns*	10.5 *±0.27*	10.4 *±0.27*	*ns*
b) Selected communities										
Monoculture average		10.3 *±0.17*	8.8 *±0.17*	***	9.4 *±0.14*	8.6 *±0.14*	***	9.8 *±0.12*	8.7 *±0.12*	***
six-species centroid		12.1 *±0.23*	10.7 *±0.23*	***	11.6 *±0.19*	10.8 *±0.19*	***	11.8 *±0.15*$^{\#}$	10.7 *±0.15*$^{\#}$	***
four-species Grass-Legume		12.3 *±0.42*	10.6 *±0.42*	***	11.3 *±0.35*	10.4 *±0.35*	***	11.8 *±0.28*$^{\#}$	10.5 *±0.28*$^{\#}$	***
four-species Grass-Herb		10.9 *±0.42*	9.9 *±0.42*	***	10.4 *±0.35*	9.7 *±0.35*	**	10.7 *±0.28*	9.8 *±0.28*	***
four-species Legume-Herb		11.7 *±0.42*	10.1 *±0.42*	***	11.7 *±0.35*	10.9 *±0.35*	**	11.7 *±0.28*$^{\#}$	10.5 *±0.28*$^{\#}$	***
five-species (excluding *L. perenne)*		12.3 *±0.26*	10.4 *±0.26*	***	11.8 *±0.22*	10.9 *±0.22*	***	12.0 *±0.17*$^{\#}$	10.8 *±0.17*$^{\#}$	***
five-species (excluding *P. pratense)*		11.9 *±0.25*	10.4 *±0.25*	***	11.5 *±0.21*	10.8 *±0.21*	***	11.7 *±0.17*$^{\#}$	10.6 *±0.17*$^{\#}$	***
five-species (excluding *T. pratense)*		11.6 *±0.26*	10.4 *±0.26*	***	11.4 *±0.22*	10.7 *±0.22*	**	11.5 *±0.18*$^{\#}$	10.4 *±0.18*$^{\#}$	***
five-species (excluding *T. repens)*		11.9 *±0.26*	10.5 *±0.26*	***	11.1 *±0.22*	10.3 *±0.22*	***	11.5 *±0.18*$^{\#}$	10.4 *±0.18*$^{\#}$	***
five-species (excluding *C. Intybus)*		12.6 *±0.26*	10.8 *±0.26*	***	11.8 *±0.22*	11.0 *±0.22*	***	12.2 *±0.18*$^{\#}$	10.9 *±0.18*$^{\#}$	***
five-species (excluding *P. lanceolata)*		12.1 *±0.26*	10.8 *±0.26*	***	11.4 *±0.26*	10.4 *±0.26*	***	11.7 *±0.18*$^{\#}$	10.6 *±0.18*$^{\#}$	***

Comparison of species' yields

The species identity estimates (Table 2.1a) can be interpreted as the monoculture performance for each species; e.g., the annual yield of a *L. perenne* monoculture under rainfed conditions in 2018 was 9.2 t ha^{-1}. The species' identity effects also feed into prediction for mixture communities as a weighted average of the component species (Fig. 2.1); e.g., the identity contribution of *L. perenne* to a mixture under rainfed conditions in year 1 is 9.1 x P_{Lp} t ha^{-1}, where P_{Lp} is the sown proportion of *L. perenne* in the mixture.

Under rainfed conditions, the highest-yielding monoculture was *T. pratense* (11.7 t ha^{-1}) in 2018 and *P. lanceolata* (10.5 t ha^{-1}) in 2019 (estimated by the species identity terms in Table 2.1a, and see Fig. 2.3). Under drought, the highest-yielding monoculture was 300N *L. perenne* in both years (10.4 t ha^{-1} in 2018 and 2019), followed by *P. pratense* (10.1 t ha^{-1}) in 2018 and *P. lanceolata* (10 t ha^{-1}) in 2019.

In 2018, the two legumes (*T. pratense* and *T. repens*) and *P. lanceolata* were the monocultures most affected by drought (annual yield reduced by 24%, 18% and 17% respectively, $P < 0.001$ in each case), while *C. intybus* and 300N *L. perenne* were much less affected (reduced by 1% and 3% respectively, n.s. in both cases). In 2019, the impact of drought on annual yield was generally lower than in the previous year, reducing yield by 0.8 t ha^{-1} instead of 1.5 t ha^{-1} in 2018. Different species were affected, with *L. perenne*, *P. lanceolata* and 300N *L. perenne* not showing a significant difference between rainfed and drought.

Functional group interactions promoted higher mixture yields under both rainfed and drought conditions

All pairwise interactions involving two species from different functional groups (grass-legume, grass-herb, legume-herb) were strong and positive in each year (Table 2.1a, see FG interaction estimates); those involving legumes were particularly strong. None of the interactions between species from the same FG were significant. However, the legume-legume and grass-grass interactions were positive in each year. The herb-herb interaction in 2018 was the only negative one, but was positive in 2019.

To isolate the effect of plant interactions on annual yield, we quantified the net effect of the interactions on yields for selected communities. We present them as the difference between predicted yield for a mixture and the weighted average yield of the component monocultures (Fig. 2.4). Mixtures including two functional groups or more, with balanced species' proportions, had a consistent positive interaction effect, ranging from +0.8 t ha^{-1}, to +2.4 t ha^{-1}, depending on year and community. As noted earlier, including legumes with other FGs was particularly beneficial, whether mixed with grass, herb, or both (Table 2.1a, FG interaction terms and Fig. 2.4).

The interaction effects were not impacted by drought; in the final model, water supply treatment was crossed with species identity effects, but not FG effects (eqn. 2.2). This means that although mixture yields were generally reduced by drought (Table 2.1b), this reduction was driven by species identity effects, rather than functional group interactions, which were robust regardless of the rainfed or drought treatment. Consequently, more diverse communities achieved a relatively higher benefit under drought compared to the corresponding monocultures.

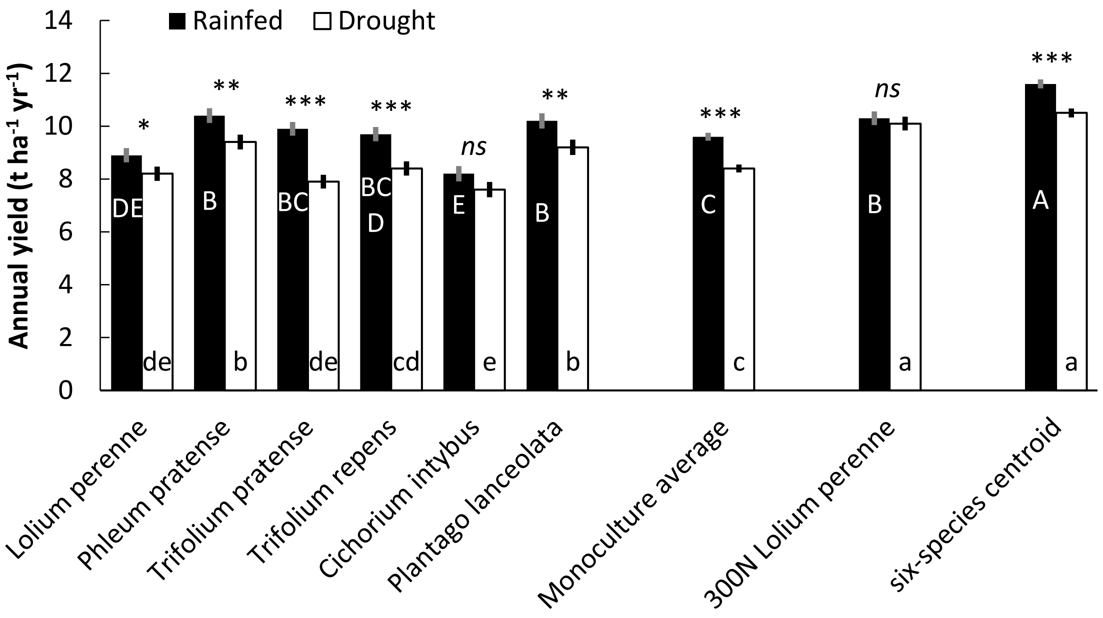
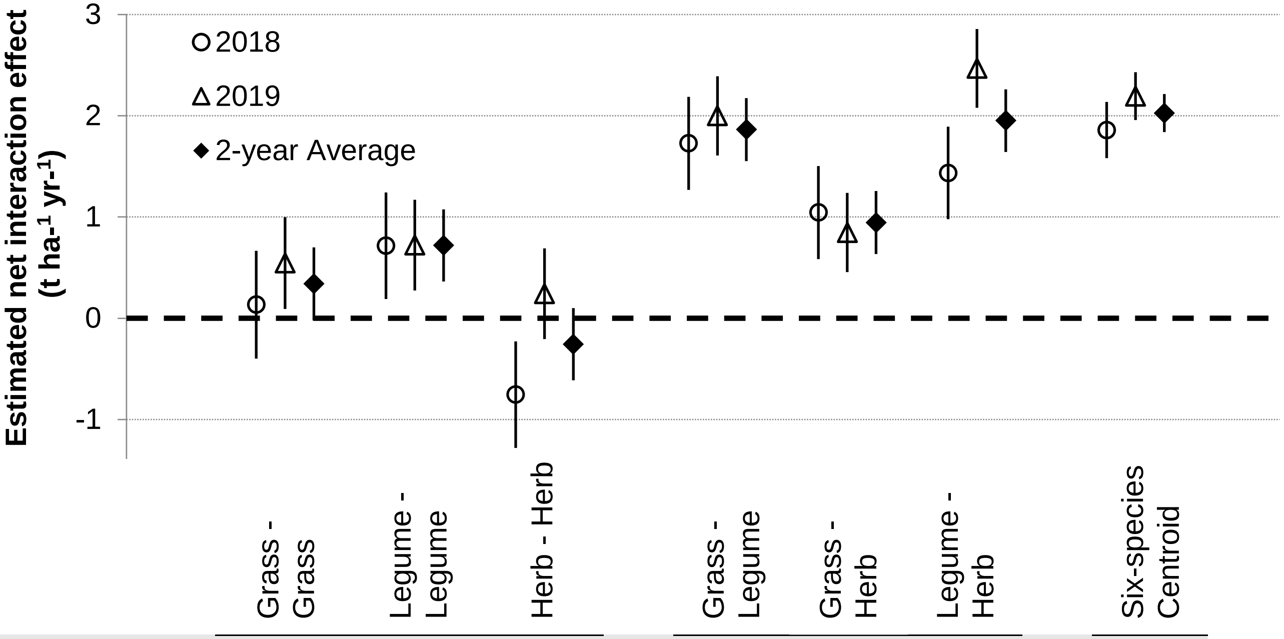


Figure 2.3. Predicted annual biomass yield on average over the two years, for low input monocultures individually, the average of all low input monocultures, the 300N *L. perenne* monoculture, and the six-species equi-proportional mixture (centroid), with standard error bars (standard errors incorporate the covariance between the two years). The significance of the difference between rainfed and drought is represented by symbols (*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns: not significant). Pairwise comparison tests were performed across the nine community types for rainfed (black bars, uppercase letters) and drought (white bars, lowercase letters) separately; within each group, bars that do not share a letter are significantly different ($\alpha = 0.05$).



Species number	2	4	6
Species relative proportions	0.5	0.25	1/6
FG number	1	2	3
FG relative proportions	1	0.5	1/3

Figure 2.4. Estimated annual interaction effect for equi-proportional mixtures over 2018 and 2019. Each value is calculated as the difference between the estimated mixture yield and the average yield of the monocultures composing the mixture. It gives the net interaction effect from the model (Table 2.1a), scaled with species and functional group proportions. Estimates and standard errors were predicted from the estimated model in eqn. (2.2). Positive values indicate a net positive interaction effect (i.e., overyielding: greater than that expected from a linear combination of the monocultures, Fig. 2.1). For communities that had two FGs, three different interaction terms are involved in the mixing effect (two within FG and one across FG). In the case of a community with three FGs, all six FG interaction terms are involved. The net interaction effect is sometimes called “overyielding effect” in literature (Nyfeler *et al.*, 2009).

Strong effects of functional group richness and composition on yield

Yield was strongly related to functional group richness and composition. Based on the average across two years, the average yields for rainfed monocultures (FG richness = 1, species richness=1) were reasonably similar over the three functional groups (9.9, 10 and 9.5 t ha^{-1},for grasses, legumes and herbs respectively, computed as the average of the identity effects of the two species within each functional group). When increasing species richness within the same FG (FG richness = 1, species richness=2), only legumes had a significant improvement in yield due to species interaction effect (Fig. 2.4, grass-grass, legume-legume and herb-herb predictions). The estimated yields of the equi-proportional four-species rainfed communities with FG richness = 2 were: grass–legume = 11.8 t ha^{-1}; grass–herb = 10.7 t ha^{-1}, and; legume–herb = 11.7 t ha^{-1} (Table 2.1b). Thus, on average over the two years, there was a clear increase in yield when FG richness increased from 1 to 2, but the magnitude of the increase was highest when legumes were included (and was thus dependent on FG composition, Fig. 2.4). The communities with two FGs accrued the interaction effects from two within-FG interactions, and one between-FG interactions. When FG richness = 3, the mixture accrued the interaction effects from three between-FG interactions and three within-FG interactions. Note that FG richness is only one dimension of diversity, and changes in relative proportions and composition can have a very large effect on performance (Fig. 2.5 and see Kirwan et al. 2009).

Using the estimates from the model (eqn. 2.2), predictions were calculated for all mixtures containing one, two or three FGs where the two species from a functional group were present in equal abundance. The predictions were displayed in ‘functional group’ ternary diagrams (Fig. 2.5). Regardless of the year or water supply treatment, communities with only one FG (in the vertices) were never the highest yielding, and there was only a slight shift in the relative abundance of FGs at peak yield (Fig. 2.5, a-f).

A wide range of mixtures achieved transgressive overyielding

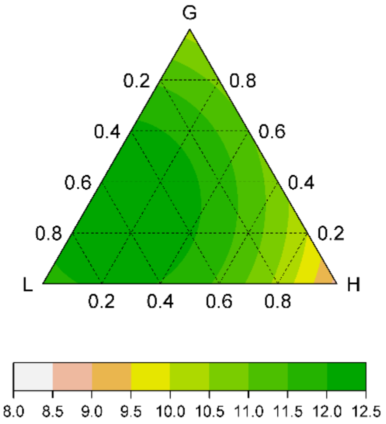
Transgressive overyielding was robust over a wide range of relative abundance of FGs, and in both rainfed and drought treatments (Fig. 2.5, g and h, on average over the two years). Averaged over the two years, transgressive overyielding was attained by all four and five-species equi-proportional mixtures, except the four-species grass-herb mixture (Table 2.1b, selected communities denoted by #).

Yield comparisons with the 300N L. Perenne monoculture

Averaged over the two years and under rainfed conditions, the 300N *L. perenne* monoculture overyielded the 150N *L. perenne* monoculture by 1.3 t ha^{-1} (10.5 – 9.2 t ha^{-1}; P<0.001, Table 2.1*)*. Simultaneously, the six-species equi-proportional mixture (centroid) overyielded the 300N *L. perenne* by 1.3 t ha^{-1} (11.8 – 10.5 t ha^{-1}; P<0.001) under rainfed conditions; thus, the high-diversity 150N community outperformed the low-diversity 300N community. Under drought conditions on average over the two years, there was a compensation of the fertiliser reduction effect by diversity. Thus, the six-species equi-proportional mixture yielded similarly under drought as the 300N *L. perenne* community under rainfed (respectively 10.7 and 10.5 t ha^{-1}, P = 0.55). In other words, the diversity effect induced by species and functional group richness at the centroid and lower nitrogen input under unfavourable drought conditions led to similar yield as that of the low-diversity, high-input comparison under favourable rainfed conditions.

Figure 2.5 (Next page). Contour plots showing the model predictions of annual dry matter yield (t ha^{-1} yr^{-1}) from any mixture sown with grass, legume and/or herb under rainfed conditions (a,c,e) or drought conditions (b,d,f). Annual yield was predicted across the 3-dimensional functional group simplex space for rainfed and drought for 2018 (a and b), 2019 (c and d) and the average of the two years (e and f). Predictions are for two species (both from one functional group – the vertices in each ternary diagram), for four species (with two species from each of two functional groups – the sides of each ternary diagram) or for all six species (three functional groups – all interior points in the ternary diagrams). For example, prediction for 100% legume is for 50% *T. pratense* and 50% *T. repens*; prediction for 40% grass and 60% herb is for 20% *L. perenne,* 20% *P. pratense,* 30% *C. intybus* and 30% *P. lanceolata;* while prediction for 10% grass, 40% legume and 50% herb is for 5% *L. perenne,* 5% *P. pratense,* 20% *T. pratense*, 20% *T. repens*, 25% *C. intybus* and 25% *P. lanceolata.* Panels g and h compare the mixture yields to best performing monoculture (*P. pratense* = BestMono) for the two-year average, taking year-to-year covariance into account. White area stands for when the predicted mixture yield is less than the best predicted monoculture (BestMono), grey area for mixture yield > BestMono, and black area for mixture yield > BestMono + 2 standerd errors (SE). Thus, the area in black displays ‘significant’ results of a conservative test for transgressive overyielding.

a)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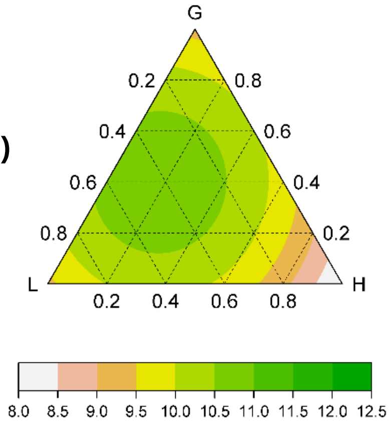
b)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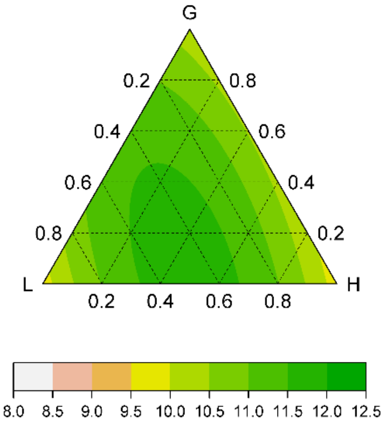
c)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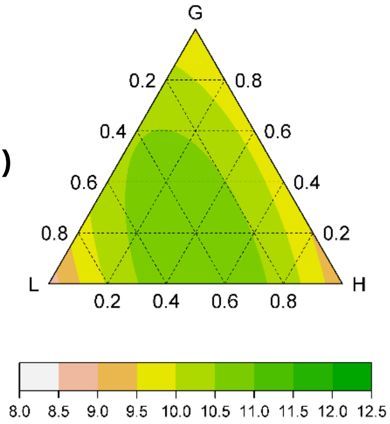
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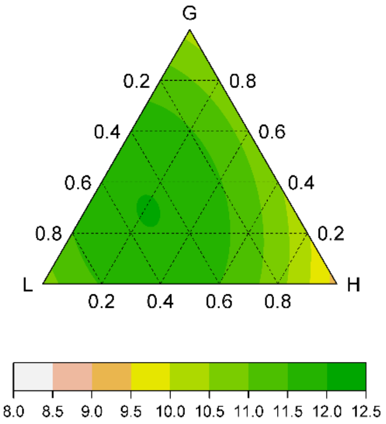
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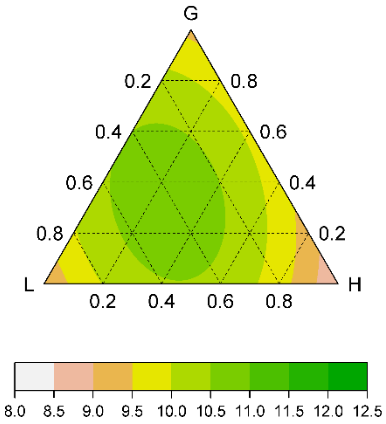
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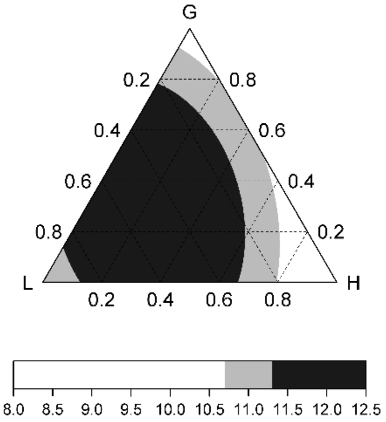
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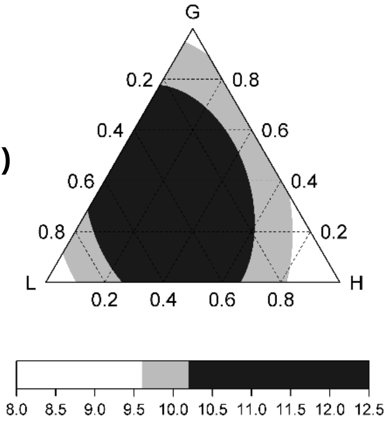
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Discussion

Overall, we found very strong effects of plant diversity on total annual yield, and this effect was maintained under experimental disturbance (drought). The additional yield due to mixing was strongly related to functional group interactions and was sufficient for mixture yields at 150N to match (under drought) or exceed (under rainfed) yields of the *L. perenne* monoculture at 300N.

Strong interaction effects between functional groups resulted in transgressive overyielding

We found strong positive effects of functional group interactions on annual yield. These interaction effects (Fig. 2.4), resulted in transgressive overyielding (outperforming the highest-yielding monoculture) for a wide range of plant communities in the design space (Fig. 2.5, g and h).

Interactions between grasses and legumes are well known to strongly increase yield (Nyfeler *et al.*, 2009; Suter *et al.*, 2015); however, inclusion of herbs in intensively managed mixtures is less understood (but see Sanderson, 2010a; Cong *et al.*, 2018; Grace *et al.*, 2018; Moloney *et al.*, 2020). Pirhofer-Walzl *et al.* (2012) showed that grass species benefited more than herbs from legume symbiotic fixations. Here, however, we show that herbs can interact with legumes as grasses do (Table 2.1a). Thus, legume-herb mixtures yielded similarly to grass-legume mixtures, on average over the two years (Table 2.1b). The grass-herb interaction effect was lower than the grass-legume and legume-herb effects, but still made a positive contribution to mixture yields. By interacting synergistically with other functional groups (grasses and legumes), herbs can contribute to transgressive overyielding.

Processes underlying the beneficial FG interactions are still to be investigated. Part of this gain can be explained by N transfer from legumes to neighbouring species. Other processes that are likely to be involved include root depth partitioning (Hoekstra *et al.*, 2014) and asynchrony of growth (Husse *et al.*, 2016; Haughey *et al.*, 2018), as well as several others (see Eisenhauer *et al.*, 2019).

Interactions between species from the same functional group are less investigated and generally overlapped by questions about species richness and composition (Hooper and Vitousek, 1998). In the present experiment, only legumes showed a consistent within-FG interaction effect. Finn *et al.* (2013) also found positive interactions between two legumes, but also between two grasses, choosing species with distant traits. Van Ruijven and Berendse (2003) confirmed that niche complementarity can increase grassland yields, even in the absence of legumes. Similar to Cong *et al.* (2018), we found an antagonistic interaction between *C. intybus* and *P. lanceolata* that did not persist in the second year. Quantifying herb-herb interactions in intensively managed grassland is relatively novel, especially under disturbance. Herbs are also associated with a variety of ecosystem services that cannot be represented by yield alone, such as nutrient recycling, reduction in greenhouse gas emissions or improvement in animal health (Wilson *et al.*, 2020). Further research is needed to optimize the multiple benefits of herb inclusion in intensively managed grasslands.

Drought effects on individual species varied widely

All six species in our study were affected by drought in at least one of the years, demonstrating the variability of monoculture yields in the face of a severe climate disturbance such as drought. Drought effect varied widely depending on the year and species. In 2018, drought effects on yield of individual species monocultures performances (150N) varied from a 1% to 24% reduction; in 2019 this varied from a 2% to 14% reduction (percentage reductions calculated from values in Table 2.1a). Over three sites in Ireland and Switzerland, Hofer *et al.* (2016) also found a wide range of species-specific drought effects on annual yield (from 6% increase to 40% reduction).

Yields of the 300N *L. perenne* monoculture were quite stable across rainfed and drought conditions and across years; the drought effect was not significant in either year. These results demonstrate that strategies to mitigate drought impacts that rely on the use of grass monocultures are unlikely to be successful, without high rates of N fertiliser (with their embedded environmental impacts).

Using a multi-model ensemble involving three different global climate models, Sweeney *et al.* (2008) predicted a reduction of 30-40% of summer rainfall in the South-East of Ireland by 2080, which corresponds to a reduction of approx. 75-100mm. The reduction in rainfall achieved by the rain shelters in this study was of same order of magnitude, with reductions of 122 and 98 mm for 2018 and 2019 respectively. The rainout shelters successfully reduced rainfall and soil moisture, but also have a combined effect on PAR, air temperature and humidity, wind speed etc. Although we refer to ‘a manipulation of water supply’, we acknowledge that the net contribution of combined changes in environmental factors will have caused the measured water supply treatment effects. Natural drought generally also goes hand in hand with variation in other changes in environmental factors, but the purpose of such study “was not to simulate a specific climate scenario, but to subject our system to severe stress to mimic extreme events that are predicted to become more frequent”, as in Hoekstra *et al.* (2015) who used a similar setup to investigate drought effects.

Practical implications

a. The impact of drought was mitigated by increasing diversity

Surprisingly, the FG interaction terms (that were predominantly strong and positive) remained the same under both rainfed and drought conditions. Their net effect meant that species interaction contribute to mitigating the impact of the extreme weather event. For example, the annual yield of the six-species centroid was 20% higher than average yields of the six monocultures under rainfed conditions, but 23% higher under drought. Knowing that drought reduced the average monoculture yield by 11%, we found a greater yield benefit from diversity than yield loss from weather disturbance in this case, as also in Finn *et al.* (2018) for four-species mixtures. In a meta-analysis of 16 temperate grassland experiments, Craven *et al.* (2016) showed that positive relationships between biodiversity and ecosystem function persisted under reduced water supply.

The effect of plant diversity on productivity and stability is well established in extensive grasslands (Isbell *et al.*, 2015), and management intensity can have a negative impact on drought resistance (Vogel *et al.*, 2012). We confirm here that plant diversity mitigated the impact of an extreme weather event in intensively managed grasslands. This is important, in the context of a predicted 12 to 40% increase in summer dry periods by 2050 (Nolan et al, 2017).

Combining observations across ten European grassland sites (nine natural and one sown) Van Sundert *et al.* (2021) analysed grassland drought effect on yield, depending on fertiliser addition. They found that graminoids were more responsive to nutrient addition than other functional types. They noted that fertiliser addition increased the negative effect of drought on biomass, especially for graminoids, while forb biomass was not affected by drought. Although we cannot directly extrapolate results from natural grasslands to intensively managed ones, a parallel can be made about the potential advantage of combining fertiliser responsive graminoids and other plant types that could balance drought sensitivity. At a larger scale, results from Van Sundert *et al.* (2021) highlight the risk associated with agricultural systems relying on fertilised grass monocultures.

b. Displacement of nitrogen fertiliser by plant diversity

In this experiment, the yield advantage from increased plant diversity was greater than the yield advantage from increasing fertiliser input from 150N to 300N. Comparing annual yield, similar biomass was produced by the equi-proportional six-species mixture in drought conditions as by the 300N *L. perenne* in both rainfed and drought conditions. Thus, manipulation of plant diversity effectively compensated for yield reductions in *L. perenne* grass monocultures due to both disturbance and lower fertiliser input.

The extra yield achieved by multi-species mixtures can be of lower environmental cost, because reducing N fertiliser results in lower greenhouse gas emissions (Velthof *et al.*, 1996). Measurement performed on the same experiment over the 2018 growth season showed a 58% reduction of N_2O emission intensity (g of N_2O per kg of dry matter yield) when comparing the six-species centroid with 300N *L. perenne* (Cummins et al., submitted).

Overall, this study demonstrates the contribution of within- and between-functional group interactions among plants in achieving higher yields, and in maintaining this contribution under conditions of environmental disturbance. Our approach also helps to clarify how changes in the sown relative proportions of the species and functional groups can affect yields under both control and disturbed conditions. Mitigating the effects of drought on grassland yields would be expected to reduce negative economic impacts (Finger et al. 2013; Schaub et al. 2020). Mixtures can also enhance multiple ecosystem functions other than yield (Dooley et al. (2015), Suter *et al.* (2021), Cummins et al. (submitted), and Grange et al. (unpublished)). We conclude that combining legumes, herbs and grasses in multi-species swards offers a practical, farm-scale management action to enhance yields in intensively managed grasslands.

Supplementary material

Table S2.1. Experimental design showing for each community the number of FG and species sown, the proportion of each, the number of replicates and the fertiliser rate (1 = 150 kg N ha^{-1}, 2 = 300 kg N ha^{-1}). ***** Due to a mistake in species proportions at establishment, this plot was removed from the design.

	Monocultures							Mixtures												
Functional group richness	1	1	1	1	1	1	1	1	1	1	2	2	2	3	3	3	3	3	3	3
Species richness	1	1	1	1	1	1	1	2	2	2	4	4	4	5	5	5	5	5	5	6
Functional group proportion																				
-Grass	1	1	1					1			0.5	0.5		0.6	0.6	0.2	0.2	0.2	0.2	1/3
-Legume				1	1				1		0.5		0.5	0.2	0.2	0.6	0.6	0.2	0.2	1/3
-Herb						1	1			1		0.5	0.5	0.2	0.2	0.2	0.2	0.6	0.6	1/3
Species proportion																				
-Lolium perenne	1	1						0.5			0.25	0.25		0.6		0.1	0.1	0.1	0.1	1/6
-Phleum pratense			1					0.5			0.25	0.25			0.6	0.1	0.1	0.1	0.1	1/6
-Trifolium pratense				1					0.5		0.25		0.25	0.1	0.1	0.6		0.1	0.1	1/6
-Trifolium repens					1				0.5		0.25		0.25	0.1	0.1		0.6	0.1	0.1	1/6
-Cichorium intybus						1				0.5		0.25	0.25	0.1	0.1	0.1	0.1	0.6		1/6
-Plantago lanceolata							1			0.5		0.25	0.25	0.1	0.1	0.1	0.1		0.6	1/6
Fertiliser rate	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Replicates	4	3	3	3	3	3	3	2	2	2	2	2	2	1	1*****	1	1	1	1	3

Table S2.2. Water supply information for the experimental site. The rainfall data was collected at Johnstown Castle weather station, at a 400m distance from the experimental field.

	2008-2017	2018	2019
Annual rainfall (mm)	1046 ± 187	1059	959
Summer rainfall (mm)	241 ± 129	100	191

Figure S2.1. Photograph of the experiment. The front of the photograph shows a main plot with a six-species community sown, with the rainfed treatment on the left-hand side and the rainout shelter to simulate the drought treatment on the right-hand side.

Chapter 3

The work presented in this chapter forms the basis a manuscript that is under review having been re-submitted by invitation at the European Journal of Agronomy. The work was carried out in collaboration with the listed co-authors; I implemented the experiment, and I led the statistical modelling work, preparation of results and writing of the paper.

Grassland legacy effects on yield of a follow-on crop in rotation strongly influenced by legume proportion and moderately by drought

Guylain Grange, Caroline Brophy, John A. Finn

Abstract

We investigated the degree to which plant species diversity, drought and fertiliser level in a grassland ley can affect performance of a follow-on crop in a rotation. Grassland species and functional group diversity (grasses, legumes and herbs) were manipulated from monocultures to six-species mixtures in the grassland ley phase. A simulated two-month summer drought treatment was compared to a ‘rainfed’ control. Plots received 150 kg ha^{-1} yr^{-1} of nitrogen (N) fertiliser; additional replicates of *L. perenne* monoculture received 300 kg ha^{-1} yr^{-1} of N fertiliser. After two years, grassland communities were terminated, and each plot reseeded with an Italian ryegrass (*Lolium multiflorum*) model crop; its yield indicated the relative legacy effect of the preceding treatments (plant diversity, drought, N input).

There was a modest but constant negative effect of drought on dry matter (-0.36 ± 0.091 t ha^{-1}) and nitrogen yield (DMY and NY respectively) of the subsequent crop of *L. multiflorum*, across all plant communities. There were strong differences among the identity effects of the six former grassland species on DMY and NY of *L. multiflorum*. Legume species had the strongest effects on DMY of *L. multiflorum* (6.09 t ha^{-1} for the former *T. pratense* monoculture and 6.54 t ha^{-1} for *T. repens*). The lowest crop yield was from the former low-diversity high-input replicates (4.16 t ha^{-1} for former *L. perenne* monoculture with 300 kg ha^{-1} yr^{-1}). There was no evidence that interspecific interactions in the grassland phase affected yield of the follow-on crop. Thus, the legacy effect of grassland mixtures was estimated by the identities and proportions of the species in the mixture. Similar patterns were obtained for NY.

High-diversity, low-input grassland yielded more DMY and NY than low-diversity, high-input grassland (across both ley and follow-on crop phases). However, a legume proportion in the grassland ley of at least 33% is required to achieve high forage and crop performance in a grassland-crop rotation.

Key-words: Crop, diversity, grassland, nitrogen, rotation, yield

Introduction

Beneficial crop rotations are increasingly important (e.g. EU Farm to Fork Strategy European Commission (2020)) to enhance the circular bio-economy and reduce significant environmental impacts associated with both imported protein crops and nitrogen fertiliser derived from fossil fuels (Lemaire *et al.*, 2015). How do we design beneficial crop rotations that best meet the multiple demands of agro-ecosystems to be economically viable and environmentally sustainable?

In a rotation, crop production depends on multiple interacting factors, some of which can be controlled by farm management (e.g. species composition of the preceding crop, rotation length, field management) (Götze *et al.*, 2017; Garland *et al.*, 2021), and others which cannot (e.g. soil type, climate, severe weather events). A variety of management decisions and environmental conditions in one part of a rotation can have persistent effects on successive crops (which we term the 'legacy effect'), and could variously result in nutrient mining, nutrient build-up and/or stimulation of soil biological functioning through plant-soil feedback (Putten *et al.*, 2016).

Intensively managed grasslands are part of rotational systems, alternating crop with grassland in crop-livestock farms (or even sequentially reseeding grasslands in livestock-only systems). Pasture-crop combinations bring agronomic and environmental benefits compared to tillage systems, improving crop yield when following a grassland, soil organic matter and water dynamics (Eriksen, 2001; Franzluebbers *et al.*, 2014; Martin *et al.*, 2020). By diversifying cultivations at field scale over time, grassland-crop rotations can help mitigate the vulnerability of intensively managed systems to extreme weather associated with climate change (Dardonville *et al.*, 2020; Emadodin *et al.*, 2021).

Here, we investigate how factors influencing yield of grassland ley (plant species composition and diversity, drought and fertiliser level) also subsequently affect the follow-on crop in a rotation. The effect of plant diversity on ecosystem functioning has been well studied, but very few experiments explore the effects of plant diversity within crop rotations. On the other hand, of studies that investigated the effect of a previous crop on the subsequent one(s), the vast majority relate to tillage rotations with rare consideration of perennial grassland leys (but see review from Martin *et al.*, 2020). Of the latter, most articles reported that plant diversity (of grassland leys and cover crops) and especially legume inclusion, lead to increased agronomic, environmental or economic benefits in crop rotations (Smith *et al.*, 2014; Nemecek *et al.*, 2015; Reckling *et al.*, 2016; Hegewald *et al.*, 2018). Rasmussen *et al.* (2012) quantified the nitrogen transfer from legumes to grass companion species and follow-on crop. Following this study, Fox *et al.* (2020b) showed that the legume proportion of an intensively managed grassland underpinned the biomass and nitrogen yield (DMY and NY respectively) response of a following crop, "with previous legume proportions of 50% having a comparable effect compared with that of a previous legume monoculture."

Regarding the influence of plant diversity on legacy effects, it is possible to envisage scenarios where the configuration of species within and among rotations could have a negative, neutral or positive impact on legacy effects, and on the total productivity of a rotation that has multiple crop combinations. For example, plant diversity can increase biomass production in grasslands (Nyfeler *et al.*, 2009; Finn *et al.*, 2013; Grange *et al.*, 2021) and so could enhance the yield of a following crop through increased belowground biomass and microbial activity that have the effect of increasing organic matter and available nitrogen for a follow-on crop (Eisenhauer *et al.*, 2017). As a contrasting example, higher DMY and NY from grassland mixtures can be partly explained by better use of available resources (Eisenhauer *et al.*, 2016), which could result in lower levels of nutrients being available for the following crop. Grassland species can also have a strong impact on soil biology even after converting to a crop (Crotty *et al.*, 2016). Thus, depending on the net outcome of these processes, the effect of plant diversity on the legacy effect of a grassland on a subsequent crop could conceivably be positive, negative or null. The potential long-term effect of weather disturbance could also interfere in the magnitude of the legacy effect of a grassland on a subsequent crop.

However, the legacy effect of grassland leys remains poorly studied beyond legume species, leaving three significant knowledge gaps. First, Fox *et al.* (2020b) study was one of the few to manipulate functional group composition and observe the effects on a follow-on crop. Focusing on the legume effect, their study represented each of the grass and herb functional groups by one species. There is a need to widen interpretations by including extra non-legume species, in order to assess the effects of within- and across-functional group interspecific interactions in grassland leys on a following crop.

Second, compared to the potential strong effect of legumes on legacy effects, the relative legacy effect of higher fertiliser levels in grassland systems remains unclear. Mineral fertiliser application remains the most common intensification practice but raises concerns about its environmental impact. Comparing high-diversity and low-input management to a low-diversity and high-input reference reveals the potential of plant diversity to enhance legacy effects. Third, it is unclear how environmental disturbances interact with the above factors (plant diversity and fertiliser application) to modify legacy effects. Extreme weather events associated with climate change (Houghton *et al.*, 2001) are a pressing concern for agricultural systems, and the potential impact on legacy effects within rotational systems is unclear (Kollas *et al.*, 2015).

We addressed these knowledge gaps in a three-year field experiment that included combinations of six species (two grasses, two legumes and two herbs) to improve understanding of the effect of plant diversity in a grassland ley on the performance of a follow-on model crop of Italian ryegrass (*L. multiflorum*). (Fig. 3.1). In addition, the experiment investigated the effect of summer drought (crossed with plant diversity) and fertiliser increase (for low plant diversity) during the grassland ley on the subsequent crop yield. Thus, we jointly studied the effect of plant diversity, drought and fertiliser increase on the legacy effect measured as performance of a follow-on model crop. The specific aims of this study were to:

- Investigate the effect of grassland diversity (from monocultures to six-species combinations) on the performance of a follow-on model crop of Italian ryegrass (*L. multiflorum*).
- Quantify the effect of an extreme weather event (drought) on legacy effects in a grassland-crop rotation.
- Compare the legacy effects of a range of grassland mixtures at low nitrogen fertiliser rate, with the legacy effect of a high-nitrogen reference level with low plant diversity.
- Identify beneficial practices in grassland management to enhance yields in both a follow-on crop and a full crop rotation.

Materials and Methods

Overview of experiment

As part of a three-year crop rotation study, we established a multi-species grassland experiment (grassland phase) that manipulated plant diversity, water supply (rainfed, drought) and included a high nitrogen treatment of *Lolium perenne* monoculture for comparison. Plot performance during the grassland phase was measured for two full growing seasons. Retaining the same spatial structure of the plots, we established an Italian ryegrass (*Lolium multiflorum*) monoculture as a model crop (crop phase) on each plot, and measured Italian ryegrass performance on the plots for one season. We define the legacy effect as the net effect of the prior treatments during the grassland phase on the subsequent performance of Italian ryegrass.

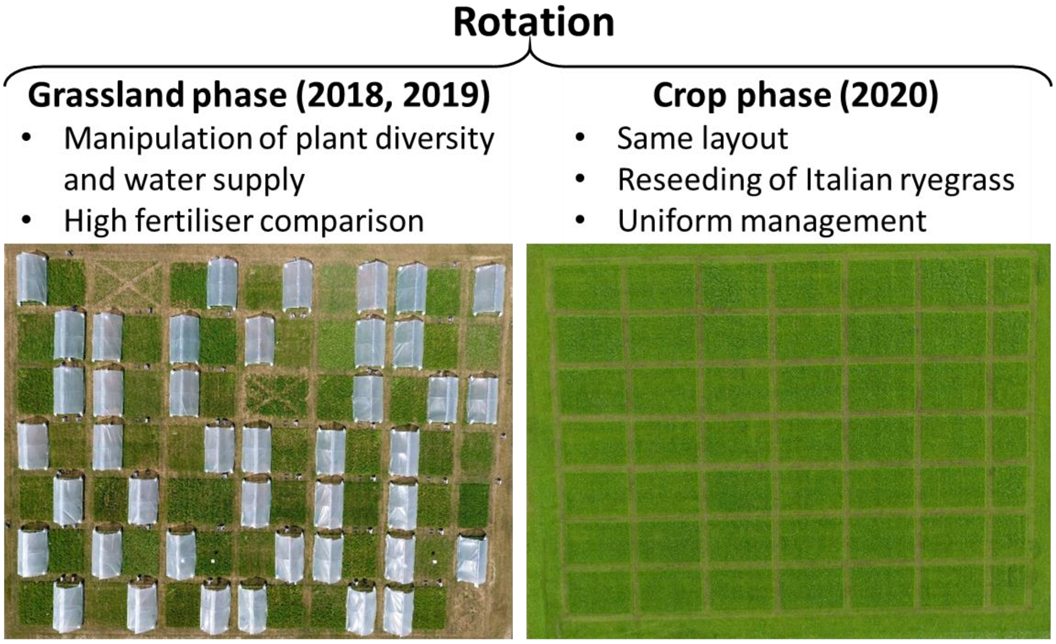


Figure 3.1. Drone image overview of the experimental design and plot management to track the effect of plant diversity, drought and fertiliser level on the legacy effect within plots. First, we manipulated grassland diversity and water supply during a two-year grassland phase and included a ‘high nitrogen fertiliser, low diversity’ reference treatment (left). We then terminated the grassland plots to establish a *L. multiflorum* (Italian ryegrass) monoculture as a model crop, keeping the same field layout and with uniform management across plots (right). A split-plot design was used; in the grassland ley phase the rain shelters (that were used to simulate drought conditions) were randomly assigned to one half of each main plot and are visible in the grassland phase image.

Grassland phase

A multi-species grassland experiment was established in April 2017 (formerly a permanent pasture of perennial ryegrass on a sandy loam soil of stagnic brown podzolic type (Baxter, 2007)). A six species pool from three functional groups (FGs) was used to create 19 different plant communities of one to three FGs. These communities comprised monocultures and mixtures of grass (*L. perenne* and *Phleum pratense*), legume (*Trifolium pratense* and *Trifolium repens*) and herb species (*Cichorium intybus* and *Plantago lanceolata*), and followed a simplex design (Cornell, 2011) across 39 main plots (Table S2.1). These plots received an annual fertiliser rate of 150 kg ha^{-1} of nitrogen (150N), 60 kg ha^{-1} of phosphorus and 300 kg ha^{-1} of potassium. Four extra replicated plots of *L. perenne* had a double rate of nitrogen (300N) applied, as a high N comparison. The location of the 43 main plots was randomised and each 7m x 5m main plot was split into two 3.5m x 5m split-plots, and each half was randomly assigned to receive either a ‘rainfed’ control or ‘drought’ water supply treatment. In each of 2018 and 2019, the rainfed control sub-plots received normal water supply (extra water supply was added through irrigation in 2018 for control to reach standard rainfall pattern), and the drought sub-plots were covered with a rainout shelter for two months, in order to simulate two consecutive years of summer drought. In order to assess the effect of rainout shelters on light availability, photosynthetically active radiations (PAR) were measured inside and outside each shelters, twice during the drought application period. The measurement were performed above the canopy using a ceptometer (AccuPAR LP80, Decagon Devices, USA). During each of 2018 and 2019, all plots were harvested on seven occasions, cutting the central strip of each sub-plot (1.5m x 5m) at 4.5cm height, to simulate grazing. See Grange *et al.* (2021) for results of the dry matter yield measurements recorded during the grassland phase, showing that increased plant diversity enhanced yield and resilience to drought.

Crop phase

All of the grassland plots were reseeded in April 2020 to establish an Italian ryegrass (*L. multiflorum*) monoculture as a follow-on model crop, keeping the same main- and split-plot locations of the grassland phase. To do so, the experimental field was sprayed with herbicide and the top 5 cm tilled with a power harrow. All plots of the follow-on crop received a total of 40 kg ha^{-1} of N in two applications of urea. Although the 43 main plot and split-plot structure was maintained, neither the experimental drought nor high N level treatments were applied during the crop phase. All plots were treated exactly the same, and only differed in the preceding treatments of the grassland phase: plant diversity, water supply level and nitrogen level. We tested the effect of these prior treatments on the Italian ryegrass monoculture by measuring aboveground DMY from four harvests during the 2020 growing season (Fig. S3.1). Dry matter was then analysed using NIRS technology to assess nitrogen content, organic matter digestibility and metabolisable energy. The same calibration as published in Lorenz *et al.* (2020) was used. Nitrogen yield (NY) was determined by multiplying the DMY by its nitrogen content. We emphasise that the DMY and NY from the follow-on crop is used to estimate the relative effects of plant diversity, drought, and 300N on legacy effects.

Statistical analysis

Results from the grassland phase were published in Grange *et al.* (2021); here we analyse measurements from the follow-on crop phase. Crop total DMY and NY of Italian ryegrass across the four harvests were analysed separately using a Diversity-Interactions model

(Kirwan *et al.*, 2009) presented in eqn. 3.1: $y = \left(\sum_{i=1}^{6} \beta_i P_i + \sum_{\substack{i,j=1 \\ i<j}}^{6} \delta_{ij} P_i P_j + \gamma X_N \right) * Drought + \varepsilon$

(Eqn. 3.1)

Where y is the subplot-level performance of the following crop of Italian ryegrass (either DMY or NY), and the predictors are the sown species proportions in the former grassland phase (P_i), with β_i the 'identity effect' for the i^{th} species. Species interactions are modelled through pairwise interactions effects (δ_{ij}), multiplied by the product of the species concerned (P_iP_j). An additional term, γ, represented the effect of increased nitrogen fertiliser rate for the replicates of *L. perenne* monocultures during the grassland phase. The factor X_N was coded 0 and 1 for plots that received 150N ($X_N = 0$) and 300N ($X_N = 1$) fertiliser respectively. Only *L. perenne* monoculture plots received the high N treatment; but for these subplots P_i was coded 0, thus, γ is the expected y of high N *L. perenne* monocultures. All predictors were crossed with a factor representing the effect of the summer drought simulated during the grassland phase (*Drought*). Thus, each of the terms β_i, δ_{ij} and γ has two estimates: one for rainfed conditions and the other for drought. Models with gradually decreasing complexity were compared to each other using AICc (i.e. drought as an additive factor not interacting with other terms, or absent; interactions simplified or absent; and redundancy of species within functional groups). Details of the range of models tested are presented in Table S3.1.

We model the crop performance on sown proportions because the sowing event is the primary manipulation of proportions in the design of a grassland community, whether for experimental purposes, or as part of a farming system. Sown and realised proportions averaged across mixtures are displayed in supplementary material (Fig. S3.2).

Results

Here, we report the effect of the treatments applied in the preceding grassland phase (species diversity, water supply treatment and fertiliser level) on the follow-on crop performance. After the model selection process detailed in Table S3.1, the best model for both responses (total dry matter yield (DMY) and nitrogen yield (NY)) was:

$$y = \sum_{i=1}^{6} \beta_i P_i + \gamma X_N + \eta X_D + \varepsilon$$

(Eqn. 3.2)

Drought was included as an additive factor and coded as a dummy variable with either $X_D = 0$ for split-plots treated as rainfed or $X_D = 1$ split-plots where drought was simulated during the grassland phase. For both total DMY and NY, the best fitting model included no interaction effects (i.e. no evidence of any $\delta_{ij} \neq 0$, eqn. 3.1). Thus, we found no evidence of synergistic or antagonistic interaction effects between grassland species affecting the following crop performance. Consequently, the final Diversity-Interactions model included species identity effects, and an additive effect of the drought factor (eqn. 3.2). The 300N treatment (X_N) for *L. perenne* monoculture was also not crossed with drought in the final model. Given the way the dummy variables are set up, γ is the expected *y* for rainfed 300N *L. perenne* monoculture, and $\gamma + \eta$ is the expected *y* for drought 300N *L. perenne* monoculture. The legacy effect of any community, including mixtures at 150N, is predicted from the weighted average of the identity effects of its component species, plus the effect for drought level. Thus, the fixed effects in eqn. 2 can be expressed to two ways, plugging in $X_N = 0$ for any monoculture or mixture at 150N gives: $\sum_{i=1}^{6} \beta_i P_i + \eta X_D$, and plugging in $X_N = 1$ for 300N *L. perenne* monoculture gives: $\gamma + \eta X_D$ (since all P_i coded 0 here). (It would be inappropriate to extrapolate the 300N effects for communities other than *L. perenne* monoculture).

Table 3.1. Model estimates and standard errors of the dry matter and nitrogen yield of *L. multiflorum* for the growing season (four harvests) (a) for the separate effects of previous grassland sown composition, fertiliser level and drought treatment (eqn. 3.2), and (b) model predictions for selected examples of mixtures (rainfed conditions). Drought had a significant ($P < 0.001$) and negative constant effect, and affected the yield of each community with the same magnitude.

(a) Model estimates	**DMY (t ha^{-1})**	**NY (kg ha^{-1})**
Lolium perenne	4.52 *±0.196*	96.9 *±4.59*
Phleum pratense	5.09 *±0.192*	105.6 *±4.49*
Trifolium pratense	6.09 *±0.196*	133.9 *±4.58*
Trifolium repens	6.54 *±0.196*	142.0 *±4.58*
Cichorium intybus	5.45 *±0.196*	115.4 *±4.58*
Plantago lanceolata	4.80 *±0.196*	97.9 *±4.58*
300N *L. perenne*	4.16 *±0.196*	88.9 *±4.58*
Drought	-0.36 *±0.091*	-9.4 *±2.28*
(b) Examples of mixtures - rainfed	**DMY (t ha^{-1})**	**NY (kg ha^{-1})**
4-species grass – legume (equi-proportional)	5.56 *±0.091*	119.6 *±2.17*
4-species herb – legume (equi-proportional)	5.72 *±0.092*	122.3 *±2.19*
4-species grass – herb (equi-proportional)	4.97 *±0.092*	104.0 *±2.18*
6-species (equi-proportional)	5.42 *±0.075*	115.3 *±1.81*
6-species (80% legume, 10% grass, 10%herb)	6.05 *±0.105*	131.2 *±2.49*

Strong identity effects and no effect of interspecific interactions on crop yield under rainfed conditions

Among the six grassland species monocultures tested, the two legume species resulted in the highest dry matter and nitrogen DMY and NY of Italian ryegrass in the crop phase (Table 3.1 and Fig. 3.2). Under rainfed conditions, former grassland monoculture of *T. repens* resulted in the highest DMY of Italian ryegrass, followed by *T. pratense* (6.54 and 6.09 t ha^{-1} respectively). Among the non-legume species, *C. intybus* was the best performing one, leading to 5.45 t ha^{-1} of Italian ryegrass biomass, under rainfed conditions. Results for the NY followed the same trend as DMY, with legume species delivering the highest legacy (142 and 134 ±4.58 kg of N ha^{-1} against an average of 104 kg of N ha^{-1} for the non-legume species).

No evidence of interspecific interactions (eqn. 3.2) meant that mixtures were predicted as the weighted average of component species. The examples given in Table 3.1 illustrate the importance of the proportion of legume in the grassland phase to enhance the yield of the follow-on crop. For example, the DMY of Italian ryegrass monoculture was 0.63 t ha^{-1} ($P<0.001$) higher if the preceding grassland ley was sown with 80% legume instead of 33%, although both contained the same six species. Similar differences were observed between equi-proportional mixtures of four species, whether including legumes or not. The effect of sown legume proportion in grassland mixtures on the yield of the follow-on Italian ryegrass crop is displayed in Fig. 3.3b.

Crop dry matter and nitrogen yield affected by drought and nitrogen level

During the grassland phase, the total amount of rainfall removed by the rainout shelters was 122 and 98 mm for 2018 and 2019 respectively and PAR was reduced by approximately 25%. However, soil moisture content in the drought treatment was restored to that of the rainfed level two months after the termination of drought (by mid-September in both 2018 and 2019), and there was considerable rainfall over the winter of 2019. Nevertheless, the experimental drought treatment in the preceding grassland phase had a constant negative effect on the legacy effect of all communities on the follow-on crop of Italian ryegrass (-0.36 t ha^{-1} and -9.4 kg of N ha^{-1} for DMY and NY respectively, $P=0.0002$). (Fig. 3.2, Table 3.1). Thus, treatments with higher DMY and NY of Italian ryegrass were proportionally less affected by drought. Summary data for metabolisable energy and digestibility of the follow-on crop are provided, but not discussed any further (Fig. S3.3).

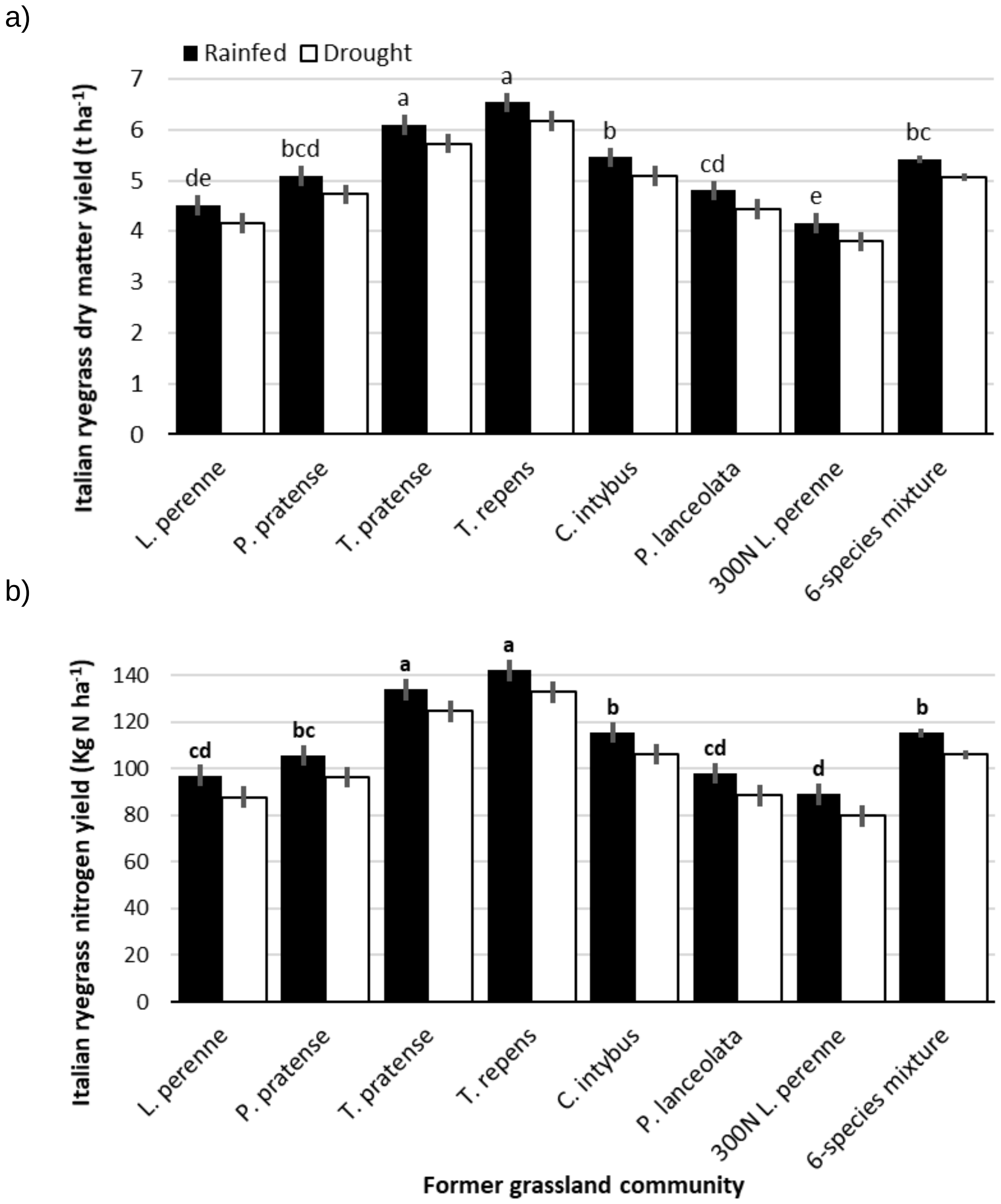
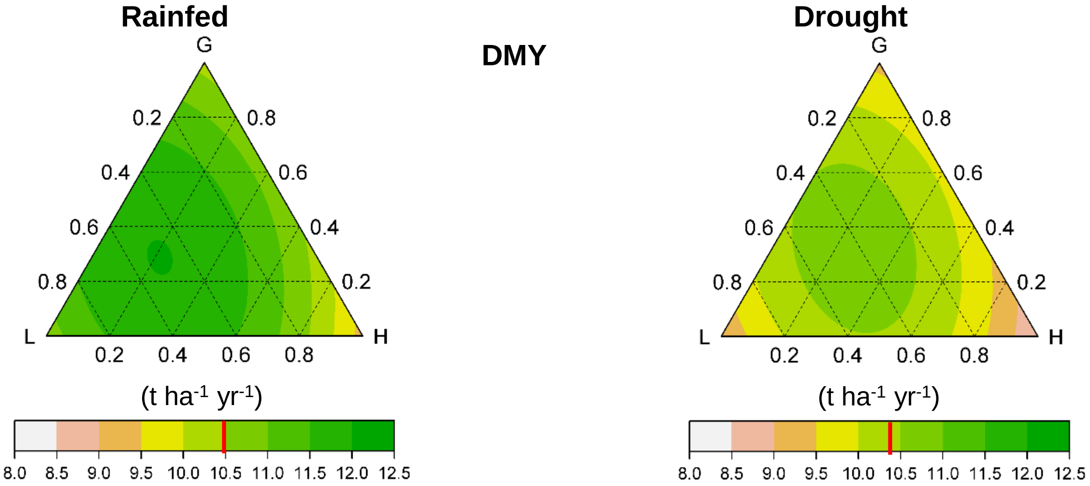


Figure 3.2. Modelled predictions from the eqn. 3.2 estimates (Table 3.1a) for dry matter (a) and nitrogen yield (b) of an Italian ryegrass (*L. multiflorum*) monoculture depending on the previous grassland plant community. Shown are the predicted monocultures for each species at 150N, the predicted 300N *L. perenne* monoculture, and the equi-proportional six-species mixture at 150N. Note that any mixture community at 150N can similarly be predicted from the model. The 300N *L. perenne* monoculture acts as a high N comparison to the regular 150N *L. perenne* community, as well as a reference for all other communities. All predictions are shown for rainfed and drought conditions. Pairwise comparison tests were performed across the displayed communities for rainfed estimates (black bars). Bars that do not share a letter are significantly different (α = 0.05). Communities that are different for rainfed (black bars) are also different for drought (white bars), as the drought effect was constant across all communities.

a) Grassland phase (average across 2018 and 2019)



b) Crop phase (2020)

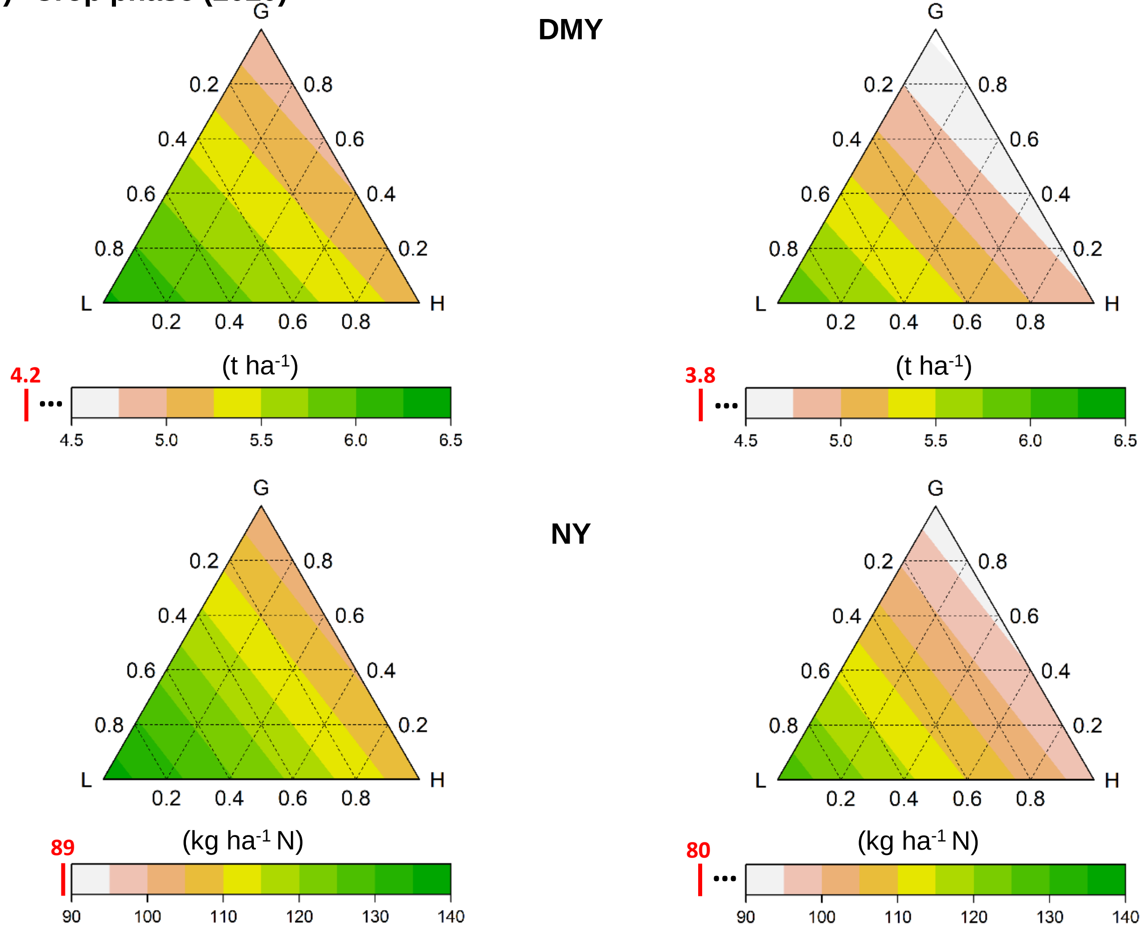


Figure 3.3. (Previous page) Effect of functional group composition (relative proportion of grasses, legumes and herbs) on average annual dry matter yield (DMY) over two years in the grassland phase (panels a; from Grange et al. 2021), and DMY and total NY (b) of *L. multiflorum* in the one-year crop phase. Predictions for the three functional groups studied are displayed with equi-proportion of the two component species (i.e. equal proportion of *L. perenne* and *P. pratense* for grass (G), equal proportion of *T. pratense* and *T. repens* for legume (L) and equal proportion of *C. intybus* and *P. lanceolata* for herb (H)). The short vertical red bars located in or beside the colour legends indicate the performance of the 300N *L. perenne* monoculture; where it is out of the legend range, the value is included in text above the red bar.

Values of DMY and NY in the crop preceded by legume monocultures were reduced by the drought, but were still higher than those for the rainfed treatment for all of the other species. Indeed, *T. pratense* under drought yielded 6.09 - 0.36 = 5.73 t ha^{-1} and 133.9 - 9.35 = 124.6 kg of N ha^{-1} respectively (Table 3.1), which are higher than any rainfed non-legume monoculture or balanced mixture. As highlighted in Section 3.1, the legacy effect of mixtures equalled the weighted average of the legacy effects of the species contained in the mixture. Because the effect of drought was constant across all species, legacy effects of mixtures were affected by drought in the same way as any other community (Fig. 3.3b).

Compared to all other communities, lowest values of DMY and NY in the crop phase were from the 300N *L. perenne* monoculture (although not significantly different from 150N *L. perenne* only; DMY (-8%, P = 0.19) and NY (-7%, P = 0.21)). The overwhelming majority of mixture communities in the ley had a significantly higher legacy effect than the 300N *L. perenne* monoculture, including mixtures without a legume contribution (e.g. Table 3.1b). For example, the equi-proportional mixture of the two species with lowest legacy effect (*L. perenne* and *P. lanceolata* at 150N) would lead to a higher legacy effect than that from 300N *L. perenne* (+0.5 t ha^{-1} from the mixture, P = 0.037). Thus, any mixture including other species from our species pool would deliver higher legacy effects than from the *L. perenne* + *P. lanceolata* example.

Treatments in the grassland phase had consistent effects across multiple harvests of crop yield

Harvest-scale raw data indicated the consistency of the treatment effects in the preceding grassland phase across the four crop harvests of *L. multiflorum* (Fig. 3.4). The DMY and NY advantage due to a preceding legume ley monoculture was evident across all harvests. In contrast, the legacy effect delivered by the high nitrogen treatment on perennial ryegrass was consistently lower than that from any other functional group or mixture.

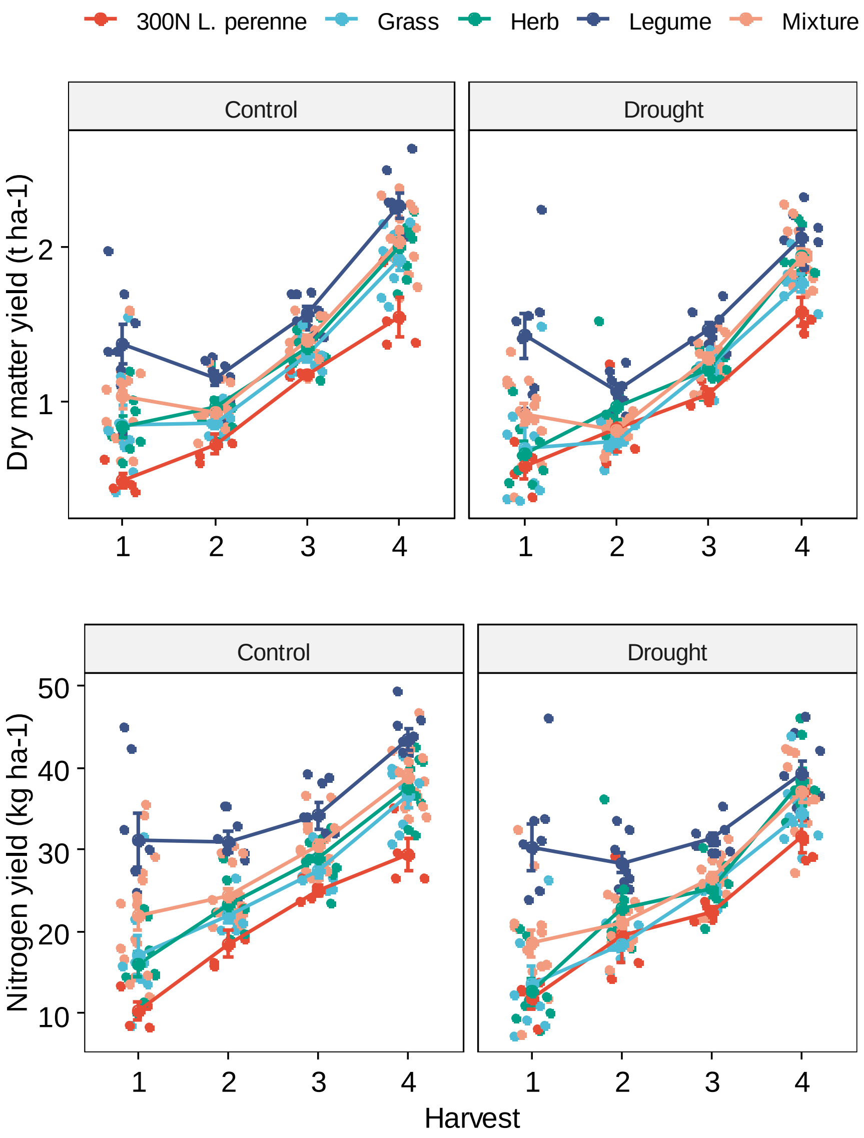


Figure 3.4. Harvest-scale raw data for DMY and NY of an Italian ryegrass crop depending on community composition of the preceding grassland (300N *L. perenne*, 150N: grass only, herb only, legume only or mixture of at least two functional groups). Bars represent standard errors. The average point for each community type is connected for each harvest to show the trends in each response over time.

Discussion

Grassland communities with higher proportions of legume species delivered higher legacy effects to the follow-on crop. Mixtures performed as the average of the component species due to the absence of plant interaction effects on the legacy effect. Experimental drought imposed during the grassland phase impacted the legacy effect by reducing DMY and NY in the follow-on crop by a constant amount (across all grassland communities).

Drought can impact yield in the follow-on crop in a rotation

In grassland experiments, rapid recovery of yield after disturbance was identified in numerous studies; once soil moisture levels are restored after experimental drought, grassland yields can show complete recovery, or even over-compensation (Vogel *et al.*, 2012; Hofer *et al.*, 2016; Haughey *et al.*, 2018; Hahn *et al.*, 2021). These studies strongly indicate high grassland resilience after drought; in contrast, we found an effect of a former drought in the subsequent year, after a change in crop within a rotation. This legacy effect of drought cannot be allocated to a remaining gradient in soil moisture as we showed in Grange *et al.* (2021) that soil moisture in drought sub-plots was back to rainfed levels two months after the end of drought simulation. In addition, all sub-plots were treated the same during the crop phase, and thus received equal water supply (weather data displayed in Fig. S3.1). It is highly likely that complex, longer-term soil processes are involved in the legacy effect of drought (which we did not directly measure in our study). The impact of climate change effect on intensively managed grasslands might be underestimated by resistance and resilience measurements that are solely restricted to a single growing season in which the drought occurs. Measurements of resilience should include more holistic and longer-term (> 1 year, at least) aspects for better estimation of the impact of disturbances such as drought.

The effect of drought on the legacy effect was constant across all plant communities. In contrast, Fox *et al.* (2020b) found no evidence of a drought effect on grassland legacy effects. This suggests that drought effects could be site-specific. Hofer *et al.* (2016) found that a very similar method to impose an experimental drought treatment in different sites resulted in differences in drought severity. They discussed the role of soil properties in affecting drought severity, and considered that sandy soils reach extreme levels of drought quicker than soils with higher amounts of silt and clay. Thus, contrasting drought impacts on grassland legacy effect across sites could be explained by soil granulometry. More research is needed to clarify the processes involved.

The long-term impact of climate change has been explored in extensive systems (Hossain and Li, 2020), but studies focusing on long-term effect of drought in intensive systems are quite rare, especially across crop rotations (but see Emadodin *et al.*, 2021). Soil drying and rewetting strongly affected humus and plant material decomposition (Birch, 1958; Lopez-Sangil *et al.*, 2018). Accelerating mineralization of organic nitrogen during rewetting can lead to important losses through N_2O emissions or leaching (Borken and Matzner, 2009; Guo *et al.*, 2014), thus reducing long-term fertility. Ploughing can also result in losses of carbon and nitrogen from soil that could cumulate with weather disturbance effect discussed above (Ball *et al.*, 2007; Willems *et al.*, 2011); in our experiment, the plots were surface tilled to avoid such an effect.

Using a multi-model ensemble involving three different global climate models, Sweeney *et al.* (2008) predicted a reduction of 30-40% of summer rainfall in the South-East of Ireland by 2080, which corresponds to a reduction of approx. 75-100mm. The reduction in rainfall achieved by the rain shelters during the grassland phase was of same order of magnitude, with reductions of 122 and 98 mm for 2018 and 2019 respectively. The rainout shelters successfully reduced rainfall and soil moisture, but also have a combined effect on PAR, air temperature and humidity, wind speed etc. Although we refer to ‘a manipulation of water supply’, we acknowledge that the net contribution of combined changes in environmental factors will have caused the measured water supply treatment effects. Natural drought generally also goes hand in hand with variation in other changes in environmental factors, but the purpose of such study “was not to simulate a specific climate scenario, but to subject our system to severe stress to mimic extreme events that are predicted to become more frequent”, as in Hoekstra *et al.* (2015) who used a similar setup to investigate drought effects.

Large differences in identity effects resulted in large diversity effects, even in the absence of interspecific interactions

There was no evidence of a net effect of grassland species’ interactions on DMY and NY of Italian ryegrass and therefore no overyielding in legacy effects (i.e. greater yield in crop preceded by mixture than weighted combination of the component species monocultures legacy effect). This absence of overyielding in legacy effect does not, however, mean that there is no effect of plant diversity on the legacy effect. The legacy effect in mixtures (in this experiment) is a weighted combination of identity effects, with large differences among species; thus, there were large differences in the legacy effect in mixtures that depended on the relative proportion of the component species (as evident in Fig. 3.3). In Fig. 3.5, we show that the six-species grassland increased the follow-on crop yield compared to a grass monoculture, as the legume content in the mixture enhanced its legacy effect. The monoculture of *T. repens* had the highest legacy effect; however, a mixture with a high proportion of legume would also deliver a high legacy effect (Table 3.1b, Fig. 3.3b).

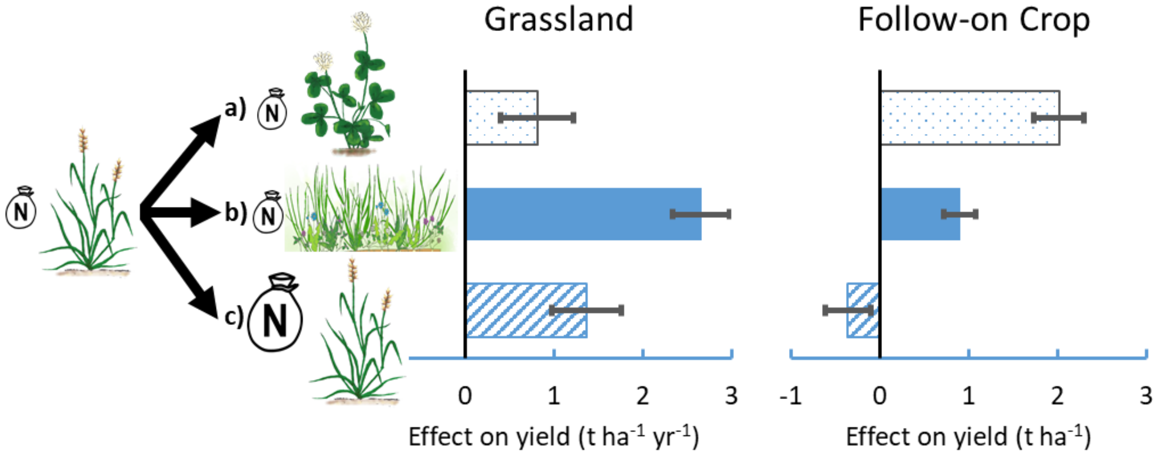


Figure 3.5. Comparison of alternative changes in grassland management practice on mean (±SE) dry matter yield in both the grassland and follow-on crop. Relative to 150N *L. perenne* monoculture (low-diversity, low-input), we compare the effect on yield across a grassland-crop rotation of (a) a 150N *T. repens* clover monoculture (the community with the highest legacy effect); (b) the six-species equi-proportional grassland mixture receiving 150N (high-diversity, low-input), and; (c) a 300N *L. perenne* monoculture (low-diversity, high-input). Using predictions from the model (eqn. 3.2), we plot the effects for scenarios (a), (b) and (c) in rainfed conditions. Data from the grassland phase is from Grange *et al.* (2021).

Using a similar experimental design and modelling approach, Fox *et al.* (2020b) found a legacy effect on DMY and NY due to synergistic interactions between legume and non-legume species (in three of four harvests). Other related studies investigated the effect of grassland diversity on processes likely to be involved in grassland legacy effects. These found no evidence of interspecific interaction effects, and mixture effects were determined solely by species' identity effects e.g. microbial activity (Fox *et al.*, 2020a) or N losses through nitrous oxide emissions (Cummins *et al.*, 2021). More research is needed for greater generality about the occurrence of synergistic interaction effects in multi-species grassland leys on a subsequent crop.

Higher nitrogen level in grassland resulted in lower yield in follow-on crop

Increased nitrogen fertiliser input in *L. perenne* monoculture grassland (300N) did not result in a higher performance of the follow-on crop in comparison to a lower fertiliser rate (150N). In addition, the extra nitrogen applied to the 300N increased NY by only 64 kg N ha^{-1} yr^{-1} during the grassland phase (unpublished). Thus, we assume that the additional nitrogen was not stored in the soil (because it did not translate into a legacy effect), and that nitrogen excess was lost from the system. In intensively managed systems, there are numerous potential sources of nitrogen loss, such as nitrate leaching and nitrous oxide emissions

(Steenvoorden *et al.*, 1986; Cummins *et al.*, 2021), with demonstrated negative impact on the environment (Ravishankara *et al.*, 2009; Leip *et al.*, 2015; Mahmud *et al.*, 2021). By managing the follow-on crop uniformly across all plots, we isolated the net effect of increased fertiliser rate on DMY and NY of the follow-on crop (Fig. 3.3b, 3.5). This approach compares the effect of two fertiliser levels in a preceding crop on the legacy effect within a rotation, which is quite uncommon (Cf. Christensen, 1997; Eriksen, 2001)

Similarly to N fertiliser rate and simulated drought, species composition effects on follow-on Italian ryegrass were still visible in the fourth (and final) harvest of the follow-on crop (Fig. 3.4), suggesting the possibility that legacy effects may persist for longer than one year (see also Fox *et al.* (2020b) for similar results). Eriksen (2001) measured an impact of a previous grassland on soil nitrogen stock and leaching three years after conversion from grassland to tillage, although the effect decreased over time. These various studies, and ours, indicate the need for a longer duration of investigation to fully measure the legacy effects of plant diversity, drought and fertiliser within intensively managed grassland-crop rotations.

Overview of both rotation phases indicates benefit of grassland diversity and legume content

In this section, we jointly discuss results from both the grassland ley phase and the follow-on crop phase in the context of the three-year rotation.

There was a strong degree of transgressive overyielding achieved by grassland mixtures in the ley phase (Fig. 3.3a, from Grange *et al.* (2021)), but overyielding due to legacy effects did not occur during the crop phase (Fig. 3.3b). One plausible explanation for such an effect is that overyielding in the grassland phase is permitted by effective resource utilisation, that could exhaust available nutrients, and result in lower subsequent DMY in the follow-on crop (Loreau and Hector, 2001; Isbell *et al.*, 2009). However, the range of community compositions associated with highest grassland DMY was not associated with any systematic or obvious reduction in DMY or NY of Italian ryegrass in the follow-on crop phase (Fig. 3.3). Thus, we observed that strong performance of species combinations in the grassland was not incompatible with strong performance in the crop phase. Smith *et al.* (2014) found similar results with annual cover-crops mixtures performing better than cover-crop monocultures, without a subsequent effect on the follow-on cash-crop. In our study, the sown proportion of the legume functional group was associated with strong synergistic effects on DMY in the grassland phase, and was also the driver of the legacy effect. Both legume content and grassland diversity are therefore critical management tools for promoting higher yields across the separate phases of the rotation.

The decision on which diversity of species (composition, richness, and proportions) to sow needs to be adapted to the agronomic objectives. This study suggests that plant diversity (approximately equi-proportional combinations of the grasses, legumes and herbs) should be favoured for higher grassland production while higher proportions of the legume functional group in grassland phase should be favoured if targeting highest legacy effects for DMY and NY of the follow-on crop. These choices are not necessarily incompatible as a high legume content in grassland mixtures would favour both forage and follow-on crop DMY and NY. Indeed, looking at DMY and NY across each of the grassland (Fig. 3.3a) and crop phase (Fig. 3.3b), the mixtures sown with ~33% to ~75% sown legume proportion delivered higher DMY than the grass and herb monocultures for either treatment. However, the decision on which non-legume species to associate with legumes in a mixture would differ depending on agronomic objectives (relative importance of outcomes for the grassland forage phase and the crop phase). Indeed, some non-legume species would deliver more yield in grassland phase, and others would deliver higher legacy effects. More investigation is needed to quantify the contribution of a wider range of legume and non-legume forage species that could favour both higher yields and environmental benefits.

High-diversity, lower-nitrogen grassland out-yielded low-diversity, high-nitrogen grassland across both ley and follow-on crop phases

The application of high levels of nitrogen fertiliser to promote higher yields is associated with several environmental impacts (Bacon, 1995). Our approach allows us to compare three different management scenarios that vary in terms of reliance on legumes only, diversity level, and fertiliser level. Using the 150N *L. perenne* monoculture (low-diversity, low-input) as a reference level for yields in both the grassland and crop phases (Fig. 3.5), we compare the effect on yield for (a) the 150N *T. repens* clover monoculture (the community with the highest legacy effect), to quantify the effect of changing species identity at the same level of input and diversity; (b) the six-species equi-proportional grassland mixture receiving 150N (high-diversity, low-input) to quantify the effect of an increase in plant diversity, and; (c) the 300N *L. perenne* monoculture (low-diversity, high-input) to quantify the effect of an increase in fertiliser use.

Relative to the 150N *L. perenne,* the legume monoculture in grassland had the greatest legacy effect; however, the legume-only yield benefit was considerably lower in the grassland phase (Fig. 3.5a) than that from the equi-proportional six-species mixture (Fig. 3.5b). Directly comparing the high-diversity, lower-nitrogen plant community with the low-diversity, high-nitrogen community across the combined grassland-crop rotation, plant diversity delivered higher performance (Fig. 3.5b), while higher fertiliser level (300N *L. perenne*) resulted in lower yields (Fig. 3.5c).

Our results are in line with those of Eriksen (2001), who showed that an unfertilized grass-clover mixture produced similar NY to a *L. perenne* monoculture receiving 300 kg ha^{-1} yr^{-1} of N fertiliser during grassland phase, but doubled the NY of the following cereal crop. That work also showed that such grassland legacy effect allowed optimal cereal DMY without any need of fertiliser in the first crop after grassland termination. In contrast, after a succession of cereal cultivations only, 115 kg of N ha^{-1} at least were needed to attain such DMY. The grassland residual effect persisted over at least three years, although it decreased over time.

Recommendations for grassland diversity and legume content depend on the relative importance of each phase of the rotation, although a diverse grassland with high legume proportion (at least 33%) would deliver high yields in both the grassland and crop phase, as illustrated in Fig. 3.3. Indicators other than yield should also be considered to refine recommendations to enhance both the agronomic and environmental performance of grassland.

In conclusion, the relative advantage of plant diversity over fertiliser in enhancing yield was evident in both the grassland and crop phases. There was a constant but moderate effect of drought across all communities in the crop phase. The use of plant diversity through legume-based multi-species grassland mixtures is a practical and effective management action to improve yields within crop rotations that contain grassland leys.

Supplementary material

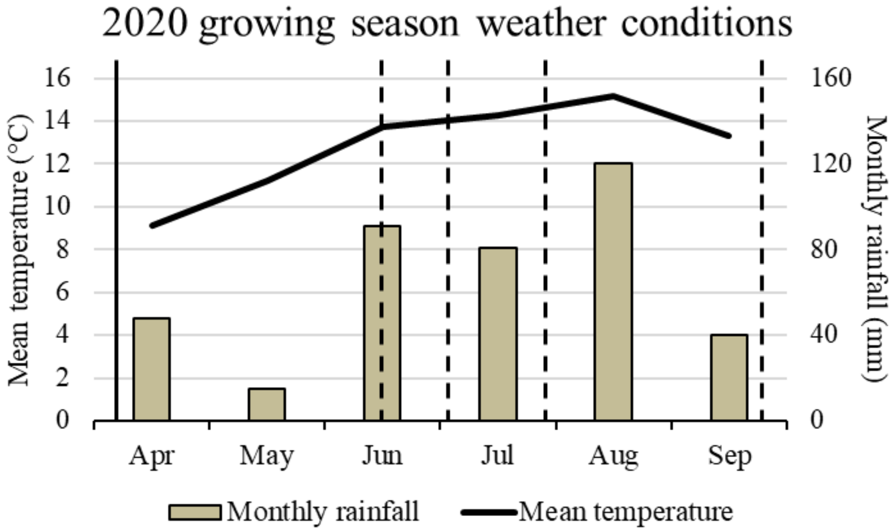


Figure S3.1. Weather conditions during the crop phase growing season. Vertical plain line highlights establishment date and vertical dashed lines show successive harvests.

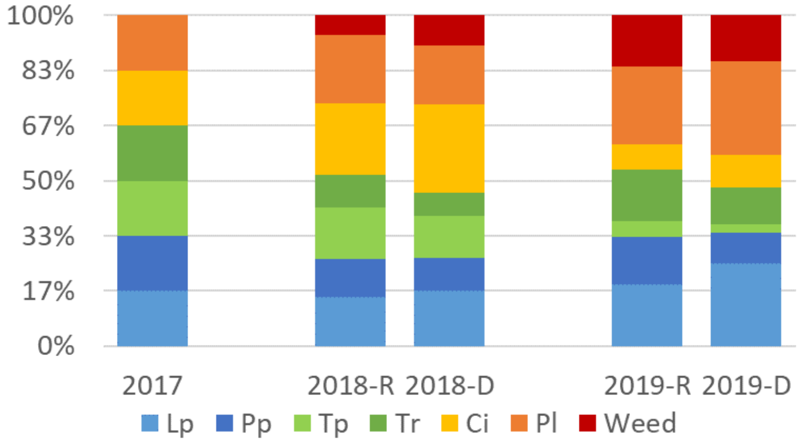


Figure S3.2. Development of species' proportions in mixtures during the grassland phase, and comparison of the rainfed control (R) and drought (D) treatments. Values for 2017 represent the sown proportions (used for modelling legacy effect). For 2018 and 2019, proportions were estimated by hand-sorting samples from each sub-plot over 3 harvests per year, and a weighted average (across all mixtures and sorted harvests) was calculated for the whole year.

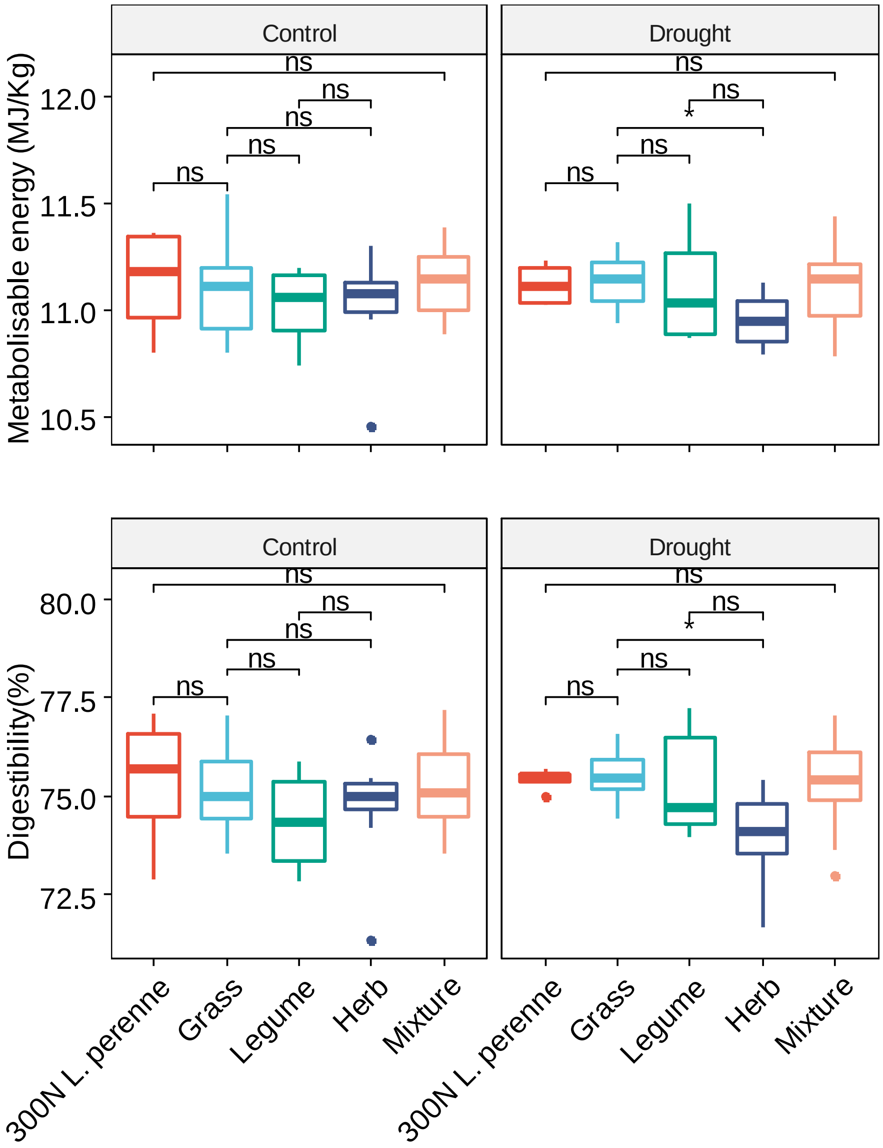


Figure S3.3. Effect of grassland composition and a two-month summer drought on the metabolisable energy and digestibility of the follow-on crop. A t-test was performed for the relevant comparisons and did not show a consistent effect across communities, except a slightly lower energy content and digestibility from a *L. multiflorum* monoculture preceded by a herb monoculture, compared to when preceded by a grass monoculture (but only after being stressed by drought). Neither of grassland water supply treatment, fertiliser increase nor plant diversity increase had a significant legacy effect on follow-on crop feeding value.

Table S3.1. Model selection process details. For both dry matter yield (DMY) and nitrogen yield (NY), models were compared across a range of fixed effects complexity. First column indicates the different plant diversity parameters tested. For each set of diversity parameters, we tested a model with either drought as an interacting effect (i.e. each diversity parameter having both rainfed and drought estimates) or as an additive effect (i.e. drought being a constant additive effect not interacting with plant diversity). The first line shows the full Diversity-Interactions model with species identities and all pairwise interactions (eqn. 3.1 in the main text shows this with drought as an interacting effect). All models were fitted with maximum likelihood for model comparisons. Comparing AICc, the model highlighted in bold was the one selected (eqn. 3.2 in the main text).

	DMY		**NY**	
Drought effect	**Interacting**	**Additive**	**Interacting**	**Additive**
Model	**AICc**	**AICc**	**AICc**	**AICc**
Species identities and interactions	140.5	119.0	683.5	661.2
FG identities and interactions	138.3	130.2	683.1	677.6
Legume, non-legume and interactions	136.1	132.2	680.2	677.6
Species identities and species richness	123.9	115.8	672.8	663.8
FG identities and FG richness	133.9	127.3	679.9	674.1
Legume, non-legume and sp. Richness	136.7	132.4	681.4	677.8
Sp. Identity and evenness	123.9	115.8	672.8	663.8
FG identity and evenness	133.9	127.3	679.8	674.0
Legume, non-legume and Evenness	136.7	132.4	681.4	677.7
Species identity	118.9	**113.8**	667.8	**661.7**
FG identity	129.2	125.3	675.2	671.9
Legume, non-legume	132.1	130.3	676.7	675.6

Chapter 4

The work presented in this chapter forms the basis a manuscript under preparation for submission to the Scientific Reports. The work was carried out in collaboration with the listed co-authors; I implemented the experiment, and I led the statistical modelling work, preparation of results and writing of the paper.

Effect of plant diversity and drought on ecosystem multi-functionality in intensively managed grassland

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Abstract

With rapidly increasing importance of environmental aspects linked to agricultural production, an approach not focused on yield alone is required for the assessment of the sustainability of farming practices. We aim to explore the relationship between plant diversity and ecosystem functioning under an intensively managed grassland-crop rotation system subject to climatic disturbance. Thus, we link the multiple facets of plant diversity with agro-ecosystem environment (climate and management) and outcomes (ecosystem functions).

We established a 3-year grassland-crop rotation experiment, manipulating six forage species from three functional groups to create plant communities of 1,2,4,5 and 6 species, following a simplex design. All plots were managed intensively for 2 years as leys (i.e. 7 cuts yr^{-1} and 150 kg N ha^{-1} yr^{-1}) and contrasting management approach was introduced as a low-diversity, high-input comparison receiving double the amount of fertiliser (*L. perenne* with 300 kg N ha^{-1} yr^{-1} (300N)). During this grassland phase, a 2-month summer drought was simulated each year on one half of each plot, the other half being a rainfed control. After two years as a grassland phase, plots were re-established with a model crop phase, with uniform management across all plots. We measured six key ecosystem functions across either one or both phases of the grassland-crop rotation (dry matter yield, yield consistency, digestibility, and weed suppression for the grassland phase, legacy effect for the crop phase, and nitrogen fertiliser efficiency for the full rotation). The functions were jointly analysed with a multivariate Diversity-Interactions model.

The relationship between plant diversity, ecosystem function and environment varied between functions, with strong positive interactions between species for half of the functions studied while the others showed no evidence of interaction effect. In general, drought reduced ecosystem functioning, except for weed suppression which was enhanced by drought for herb species. When occurring, interaction effects maintained under drought, leading to relatively higher benefit of diversity. Predicting on average across all functions in a multifunctionality index, we identified a wide range of diverse mixtures that achieved a transgressive over-performance (i.e. out-performed the best monoculture). The low-diversity, high-input comparison (300N) had a low multifunctionality index, being out-performed by a high-diversity, low-input community (150N equi-proportional six-species mixture) under both rainfed and drought conditions. We conclude that plant diversity is a practical tool to enhance multifunctionality under intensive management while mitigating the impact of drought.

Key-words: Multifunctionality, grassland, diversity, rotation, drought, transgressive overyielding.

Introduction

Agriculture is a source of multiple outcomes of great importance for humans. Along the food production process, farmers rely on the simultaneous functioning of several ecosystem functions that translate into services such as soil fertility, pest regulation, weed suppression, water purification, etc. (Manning *et al.*, 2018). Narrowing agriculture objectives to the sole aim of food production threatens the delivery of other crucial ecosystem functions (Tilman *et al.*, 2002; Zavaleta *et al.*, 2010; Tsiafouli *et al.*, 2015; Schils *et al.*, 2022). Plant diversity is generally associated with enhanced ecosystem functioning in semi-natural grasslands (Hector and Bagchi, 2007; Allan *et al.*, 2013; Isbell *et al.*, 2017). On the other hand, intensification of agriculture over the last decades led to a focus on the productivity function alone, relying on a low number of species with high yielding potential and good response to high and regular levels of fertiliser input and water supply (Hopkins and Holz, 2006).

Severe weather events are linked to a decreased delivery of ecosystem functions (Rosenzweig *et al.*, 2001; Coronese *et al.*, 2019). As climate change challenges the current and future farming systems with increased occurrence of extreme weather events (Hoegh-Guldberg *et al.*, 2018), sustainability and resilience of agriculture are becoming prominent topics. We argue that neither can be resolved with single measures of performance for agro-ecosystems that focus on production alone. Aspects other than yield need to be considered in a simultaneous assessment of multiple ecosystem functions. Can we enhance agro-environmental performances and increase resilience by diversifying intensively managed systems? Can these be jointly delivered or are there trade-offs? The work in this chapter aims to investigate the effect of plant species and functional diversity on the achievement of multiple ecosystem functions from intensively managed grasslands, in a context of weather disturbance.

To measure such effects, “multifunctionality” refers to the assessment of multiple functions simultaneously, including correlations between the functions studied. As pointed out by Suter *et al.* (2021), this concept relates to several definitions and corresponding methods. Among the published methods for multifunctionality analysis, species richness is often the sole indicator of plant diversity. The multiple threshold method suggested by Byrnes *et al.* (2014) reflects the complexity of achieving high performances across several functions simultaneously, but needs to be adapted to consider composition and relative abundance rather than the count of species. Similar limitation applies to the overlap method (Alatalo and Alatalo, 1977; Hector and Bagchi, 2007), where the richness required to optimise one or more functions is assessed.

Under intensive management, the selection of the best performing community is more important than the identification of a trend along species richness. Moreover, low species richness and intensive management increase the chances of dominance from species or FG that need to be taken into account for accurate assessment of the BEF relationship (Roscher *et al.*, 2007; Mouillot *et al.*, 2011; Bessler *et al.*, 2012; Arnillas *et al.*, 2021). For these reasons, the metric of species richness is not adapted to an assessment of the multiple facets of diversity in intensively managed agro-ecosystems, where a restricted number of targeted species is chosen and sown with specific objectives (Roscher *et al.*, 2007; Kirwan *et al.*, 2009; Brophy *et al.*, 2017a; Abalos *et al.*, 2021). Figure 1.4 (in Chapter 1) illustrates how richness alone cannot fully describe the performance of a grassland with established species from a restricted pool. We also highlight that the best performance does not necessarily correspond to the highest diversity community (*per se*, the one with highest richness and evenness).

Diversity-Interactions models as described by Kirwan *et al.* (2009), allow to isolate the contribution of species or functional group as well as their interactions to a community outcome. Applied to grasslands, this method can quantify the effect of each plant species (identity effect) as well as the effect of interspecific plant interactions on the delivery of an ecosystem function, thus helping to disentangle the biodiversity-ecosystem function (BEF) relationship. Dooley *et al.* (2015) developed a multivariate Diversity-Interactions model to jointly assess the complexity of both plant diversity and interdependent ecosystem functions. Suter *et al.* (2021) used a similar multivariate approach and deepened the analysis of the model outcomes with response ratios (Hedges *et al.*, 1999), to investigate the effect of species interactions across ecosystem functions and varying diversity.

Using the Diversity-Interactions multivariate model, we propose to assess the multivariate BEF relationship through a 3-year field experiment that manipulated plant diversity and imposed a water supply treatment with two levels (rainfed control and drought). A larger pool of species is used (six species instead of four in Dooley *et al.* (2015) and Suter *et al.* (2021)), to disentangle the contribution of each species and specific interactions between species or functional groups on multifunctionality. Herb species are included in the design with the objective to quantify their contribution to grassland multifunctionality, either in comparison or in association with legume and/or grass. Finally, as sown grassland species proportions can be managed at establishment, we relate the links between diversity and multifunctionality to the sown composition and relative abundance of species and functional groups in communities.

Multiple facets of plant diversity are thus manipulated, alongside water supply treatment and a highly fertilised low-diversity comparison. We linked them with a variety of outcomes, i.e. ecosystem functions: annual yield, yield consistency, digestibility, legacy effect, nitrogen fertiliser efficiency and weed suppression. These functions are not necessarily independent and trade-offs can appear between some of them. Also, different aspects of plant diversity can have divergent effects on a given function and/or across ecosystem functions.

Performing such study under intensive management and crossing with a disturbance is novel, so is the species pool studied with the inclusion of herbs and the ability to investigate within- and between-functional group interactions. This way, we address the following research questions:

- What is the relative importance of plant diversity on multiple ecosystem functions?
- Are there trade-offs among ecosystem functions?
- What is the impact of drought on each ecosystem function and multifunctionality across grassland-crop rotations that varied in plant diversity?

Materials and Methods

The experiment

A 3-year grassland-crop rotation experiment was carried out in Johnstown Castle, Co. Wexford, in the South-East of Ireland. A grassland phase of the rotation was established in April 2017 and plots were measured for two years following the year of sowing (2018 and 2019). The follow-on crop phase commenced in spring of 2020 and plots were measured over the growing season that year (Fig. 4.1).

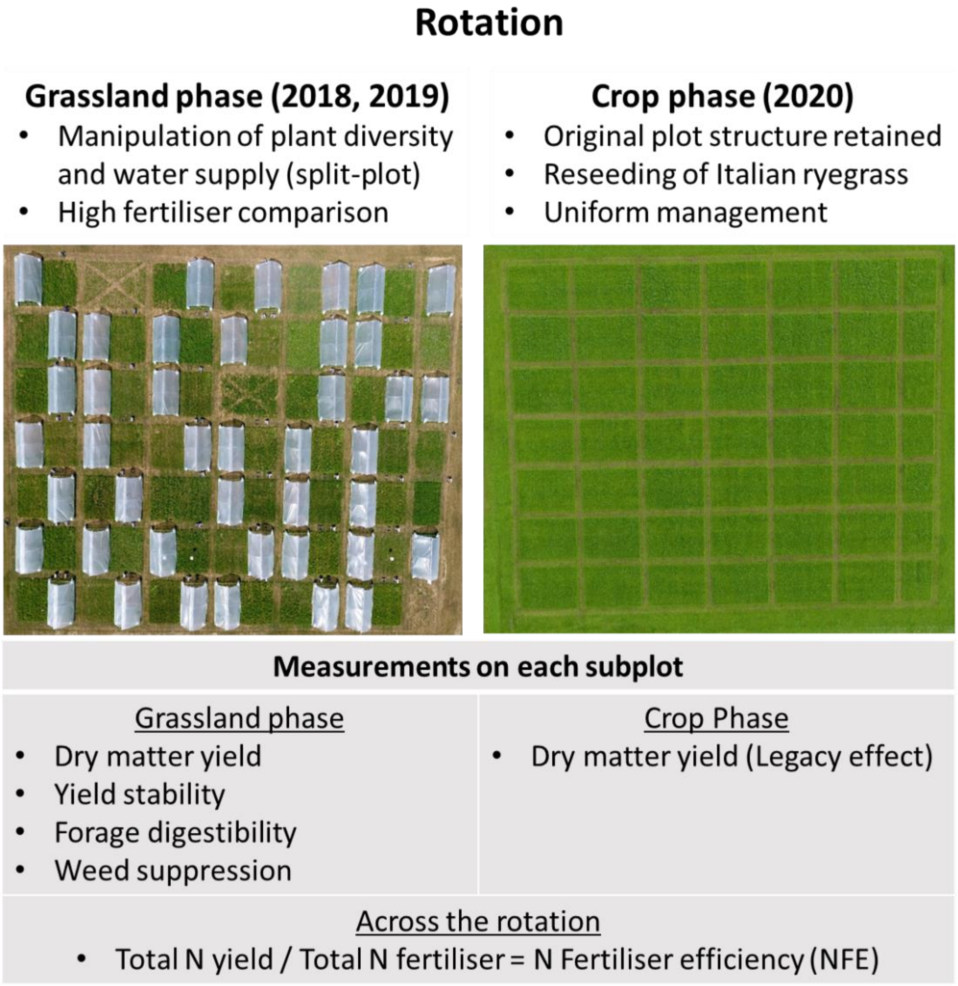

Figure 4.1. Field layout and schedule of the measurements for the experiment. The six functions measured are explained in more detail in the text.

In the grassland phase, plots of 5m x 7m were sown with varying combinations of plant species using a pool of six species from three functional groups: grass (*Lolium perenne* and *Phleum pratense*), legume (*Trifolium pratense* and *Trifolium repens*) and herb (*Cichorium intybus* and *Plantago lanceolata*). Following a simplex design, 19 plant communities were systematically assembled in a total of 39 plots, ranging from monocultures to six-species mixtures with proportions of each species varying between 0%, 10%, 25%, 50%, 60% and 100% depending on the community (see Table S2.1 for more details). In this phase, all plots were fertilised with an annual rate of 150 kg of nitrogen (N), 30 kg of phosphorus and 300 kg of potassium per hectare per year. Four extra replicated plots of a *L. perenne* monoculture received double the amount of N as a more intensive management comparison (300N), giving a total of 43 plots. To simulate the effect of a summer drought, each of the 43 plots was divided in two 3.5m x 5m split plots and the subplots were randomly assigned to either a rainfed control or a summer drought treatment. Drought was simulated by covering the drought subplots with rainout shelters for a two-month period in 2018 and 2019. Rainfall exclusion resulted in extreme drought stress for the two summers (soil water potential < -1.5MPa, see Fig. 2.2 for more detail). In order to assess the effect of rainout shelters on light availability, photosynthetically active radiations (PAR) were measured inside and outside each shelters, twice during the drought application period. The measurement were performed above the canopy using a ceptometer (AccuPAR LP80, Decagon Devices, USA).

Multiple measurements were taken from the grassland phase, through seven annual harvests, when total aboveground biomass was cut at 4.5cm height, using a Haldrup harvester. Dry matter yield was determined after oven drying samples of the material harvested. The dry samples were then ground and sent for infrared spectroscopy analysis to assess nitrogen content and digestibility of the material (using the same calibration as Loges *et al.* (2018)). For three harvests in each year, a representative sample of the freshly cut material was sorted to measure the proportion of plant biomass comprising each of the sown species and weeds.

In March 2020, the grassland phase was terminated by application of glyphosate. The plots were reseeded in April 2020 with a monoculture of *Lolium multiflorum* that served as a model crop, keeping the same experimental field layout of subplots / plots. All plots were managed uniformly in 2020 and both subplots within each plot were also treated identically, i.e. there was no manipulation of drought in the follow-on crop phase. All plots / subplots received the same fertiliser in this phase (40 kg N ha^{-1}), including the plots that received the high N treatment in the grassland phase. Homogeneous management in this phase enabled isolation of the effects of the treatments in the grassland phase (plant diversity, drought and high N) on the follow-on crop performance. Model crop biomass was cut on four occasions during 2020 growing season, and dry matter nitrogen content was assessed for each harvest.

Ecosystem functions analysed

We analysed the following six ecosystem functions that are important agronomic indicators within intensively managed grasslands for livestock production (Fig. 4.1).

From the grassland phase:

- **Dry matter yield** (DMY) was measured as the average annual yield from the two years of the grassland phase.
- The standard deviations of yield across the seven harvests in each year during the grassland phase were averaged and used as the indicator of **yield consistency**.

- **Digestibility** of the harvested forage material was averaged across the 14 grassland phase harvests (weighted by harvested biomass).
- Weed proportion was averaged across the three harvests that were sorted (weighted by harvested biomass) and **weed suppression** was calculated as 1 – weed proportion.

From the follow-on crop phase:

- The follow-on crop yield (summed across four harvests) was used as the indicator for the **legacy effect** of the grassland to the follow-on crop of *L. multiflorum*.

From the full rotation:

- Total nitrogen yield in harvested forage across the grassland-crop rotation was divided by the total amount of nitrogen fertiliser spread across both phases to calculate the overall **nitrogen fertiliser efficiency** (NFE).

Data preparation

To enable simultaneous analysis of multiple functions, we standardized all measurements to a comparable scale, following the method used in Dooley *et al.* (2015). The raw data for each function was standardized by dividing each variable by the average of its top three values. For most of the functions, high values measured corresponded to high achievement from an agronomic perspective (DMY, legacy effect, digestibility, NFE). Weed suppression was already on a scale of 0-1 and so was not standardised. For yield consistency, small standard deviation values are considered more desirable, thus, the three minimum values were used as a reference and standardizing ratio was inverted. The standardization process aims to scale the data between 0 and 1, with value = 1 being the high achievement reference for each function. In practice, some values may lie a little above 1 (on account of the division by the average of the three highest values, not the single highest); in the standardized dataset, the values across all functions ranged from 0.36 to 1.05.

Data analysis

We used the multivariate Diversity-Interactions model (Dooley *et al.*, 2015) to study the effect of plant diversity on several ecosystem functions and incorporate the covariances between functions as part of the model. The following general model structure was used:

$$y_k = \left(\sum_{i=1}^{s} \beta_{ik} P_i + \sum_{\substack{i,j=1 \\ i<j}}^{s} \delta_{ijk} P_i P_j + \gamma_k X_N \right) * Treatment + \varepsilon$$

Eqn. 4.1

Where y_k is the standardized observed value for a given function k taken on each subplot. The model parameters: species identities (β) and interactions (δ) were scaled to corresponding species proportions (P_i and P_j) as explanatory variables. The high fertiliser comparison was considered as an extra term (γ), as the high fertiliser treatment (300N) was applied to *L. perenne* monoculture replicates only (X_N=1 for 300N and 0 in other cases). The water supply treatment (drought or rainfed; denoted '*Treatment*' in eqn. 4.1) was crossed with all terms in the model. The error structure allowed for both the split plot design and the multivariate nature of the response: (1) plot was treated as random to adjust for the split plot nature of the design; (2) a block diagonal structure with a block for each subplot that included a unique variance for each function and unique covariance between each pair of functions to capture the multivariate nature of the responses. We used SAS software function 'proc mixed' for model fitting (SAS Institute, 2013).

For preliminary assessment of the structure of identity, interaction and drought effects, a range of univariate Diversity-Interactions models with varying fixed effects were fitted and compared for each function, using AICc to compare models. These function-scale univariate models informed the model selection for the multivariate model. A second round of model selection (also using AICc) was performed on multivariate model to adjust the error structure.

To investigate the performance of communities across all studied functions, we used our final model to predict the (unweighted) average across all functions for a range of communities and called it 'multifunctionality index' (MF index).

Results

Preliminary work on single ecosystem functions highlighted that for some functions the water supply treatment was only required as an additive factor, while for other functions the water supply treatment was crossed with some model terms. Similarly, species interaction terms were not relevant for all functions. We thus adapted our multivariate model to incorporate drought and species interaction effects differently depending on functions. The final model did not cross drought with species interactions for any function. Thus, the interaction effect, describing the difference between a mixture and the weighted average of the component species identities, was not affected by the occurrence of a two-month drought. The final multivariate model was adapted by grouping functions depending on the relevant type of drought and interaction effect. For DMY, NFE and weed suppression, the interactive effect of drought resulted in different estimates across water supply treatments for each identity effect and the high N comparison (eqn. 4.2.a). For the other functions (yield consistency, legacy effect and digestibility), a single value of was estimated for each species *i* (eqn. 4.2.b). A single constant drought effect was estimated across all observation for the given function *k,* and scaled by the drought treatment ($X_D = 0$ for rainfed and $X_D = 1$ for drought). No interaction effect was estimated as we found no evidence of species interaction for these functions. Final model fixed parameter estimates are displayed in Table 4.1 and the variance covariance matrix estimates are displayed in Table S4.1.

$$\hat{y}_k = \left(\sum_{i=1}^{s} \hat{\beta}_{ik} P_i + \hat{\gamma}_k X_N\right) * treatment + \sum_{\substack{i,j=1 \\ i<j}}^{s} \hat{\delta}_{ijk} P_i P_j$$

Eqn. 4.2.a

$$\hat{y}_k = \sum_{i=1}^{s} \hat{\beta}_{ik} P_i + \hat{\gamma}_k X_N + \hat{\eta}_k X_D$$

Eqn. 4.2.b

Drought effect varied across functions and communities

The total amount of rainfall removed by the rainout shelters was 122 and 98 mm for 2018 and 2019 respectively and PAR was reduced by approximately 25%. This experimental drought caused a significant reduction in ecosystem function across all functions except digestibility where the drought treatment was not significant (Table 4.1) and weed suppression where the direction of the effect was species specific (compare the identity effects under rainfed and drought for weed suppress. in Table 4.1). For the functions where the water supply treatment interacted with species identity effects (DMY, NFE and weed suppression), the effect varied among species. For DMY and NFE, *T. repens, T. pratense, P. pratense* and *P. lanceolata* had high identity effects but were the most affected by drought, while *C. intybus* and *L. perenne* had low DMY and NFE but were less affected by drought than other species for these functions. Drought (compared to rainfed) reduced weed suppression only for the *T. repens* identity effect, but enhanced weed suppression for the herb species. The most resistant species to drought across all functions was *C. intybus*, followed by *L. perenne*.

Performances show trends in functional groups

Across most functions, there were dissimilarities between species' performances (Fig.4.2). Species from the same FG often showed consistent pattern on the responses across function. The legacy effect and NFE were favoured by legume species identity effects. Yield consistency was significantly better from herb species than the average monoculture. Negative correlations between some functions (e.g. NFE and weed suppression) reflected the difficulty of performing high in those functions simultaneously. Weed suppression was higher in grass and *P. lanceolata* monocultures. Estimates of species contributions to a community were defined as $\widehat{\beta_i} * P_i$. In monocultures, performance equalled species identity effects ($\widehat{\beta_i}$) as $P_i = 1$ (cf. Table 4.1 and Fig. 4.2). Species identity effects also fit in mixtures estimates, with each species contribution scaled to it proportion (P_i) in the mixture. Thus, a mixtures highly dominated by one species would perform similarly to the monoculture of this species.

The MF index summarizes the performance of communities across functions (Table 4.1, Fig. 4.2 and 4.3). Among species' identities, *T. repens* delivered the highest multifunctionality (MF index) under rainfed conditions, closely followed by *P. lanceolata*. Under drought, *P. lanceolata* had the highest MF index. *Cichorium intybus* and the 300N *L. perenne* achieved the lowest MF index values (Fig. 4.2). We note that a mixture of 70% *L. perenne* and 30% *T. repens* had a significantly higher MF index than any monoculture, under both conditions (Table 4.1). However, it was still significantly outperformed by the six-species equi-proportional mixture (P = 0.002 in both rainfed and drought conditions).

Table 4.1. (Next page) (a) Estimates for fixed effects based on model displayed in eqn. 4.2. Drought and interaction terms allocations were chosen after model selection on single functions. Interactions and drought effect in bold are significantly different from zero (α=0.05), others are not. Differences between rainfed and drought estimates for species identities and 300N *L. perenne* are indicated as *** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, Ø P > 0.05 for the three first functions where drought was an interacting effect. For the other functions, drought was a constant additive effect (^{1}). In these case, the value displayed is the magnitude of drought effect (= difference between rainfed and drought across all identity effects, including 300N *L. perenne*), e.g. the standardized yield consistency for *P. pratense* under drought is the sum of the rainfed estimate for *P. pratense* and the drought additive effect: 0.572 + (-0.089) = 0.483.
(b) Predictions for selected communities. Values in italics (part (b) and MF index) are predicted from model parameters (a). MF index in last column displays the unweighted average prediction across functions for the species monocultures (aligned with identity effects in (a)) and selected communities (b).

	DMY		**NFE**		**Weed suppress.**		**Yield consist.**		**Legacy effect**		**Digestibility**		***MF Index***	
a) Fixed Effects	Rainfed	Drought	Rainfed	Drought	Rainfed	Drought	Rainfed	Drought	Rainfed	Drought	Rainfed	Drought	*Rainfed*	*Drought*
Species identity														
L. perenne	0.735	0.673 *	0.606	0.547 *	0.848	0.903	0.854		0.630		0.966		*0.773*	*0.739* ***
P. pratense	0.844	0.778 ***	0.731	0.667 **	0.848	0.866	0.572		0.709		0.908		*0.769*	*0.727* ***
T. pratense	0.799	0.656 ***	0.926	0.768 ***	0.467	0.472	0.861		0.849		0.916		*0.803*	*0.731* ***
T. repens	0.808	0.691 ***	0.950	0.823 ***	0.493	0.394 ***	0.778		0.911		0.922		*0.811*	*0.731* ***
C. intybus	0.679	0.635 *	0.703	0.649 *	0.455	0.540 **	0.889		0.760		0.922		*0.735*	*0.710* *
P. lanceolata	0.854	0.749 ***	0.665	0.601 **	0.819	0.893 **	0.892		0.670		0.902		*0.801*	*0.762* ***
Interaction														
Grass*Grass	0.110		0.029		-0.007									
Legume*Legume	0.135		**0.205**		0.086									
Herb*Herb	-0.016		-0.086		**0.476**									
Grass*Legume	**0.519**		**0.274**		**1.069**									
Grass*Herb	**0.270**		**0.154**		**0.588**									
Legume*Herb	**0.566**		**0.211**		**1.221**									
300N *L. perenne*	0.845	0.827	0.451	0.425	0.925	0.924	0.704		0.580		0.970		*0.746*	*0.716* **
Drought (Additive) ¹								**-0.089**		**-0.051**		0.003		
b) Selected communities														
Monoculture average	*0.786*	*0.697* ***	*0.763*	*0.676* ***	*0.655*	*0.678*	*0.808*	*0.729* ***	*0.755*	*0.704* ***	*0.923*	*0.925*	*0.782*	*0.734* ***
70% L. perenne + 30% T. repens	*0.866*	*0.787* ***	*0.767*	*0.687* ***	*0.966*	*0.975*	*0.831*	*0.743* ***	*0.714*	*0.663* **	*0.953*	*0.956*	*0.850*	*0.802* ***
6-species equi-proportional mixture	*0.943*	*0.854* ***	*0.838*	*0.751* ***	*0.99*	*1.013*	*0.808*	*0.729* ***	*0.755*	*0.704* ***	*0.923*	*0.925*	*0.877*	*0.828* ***

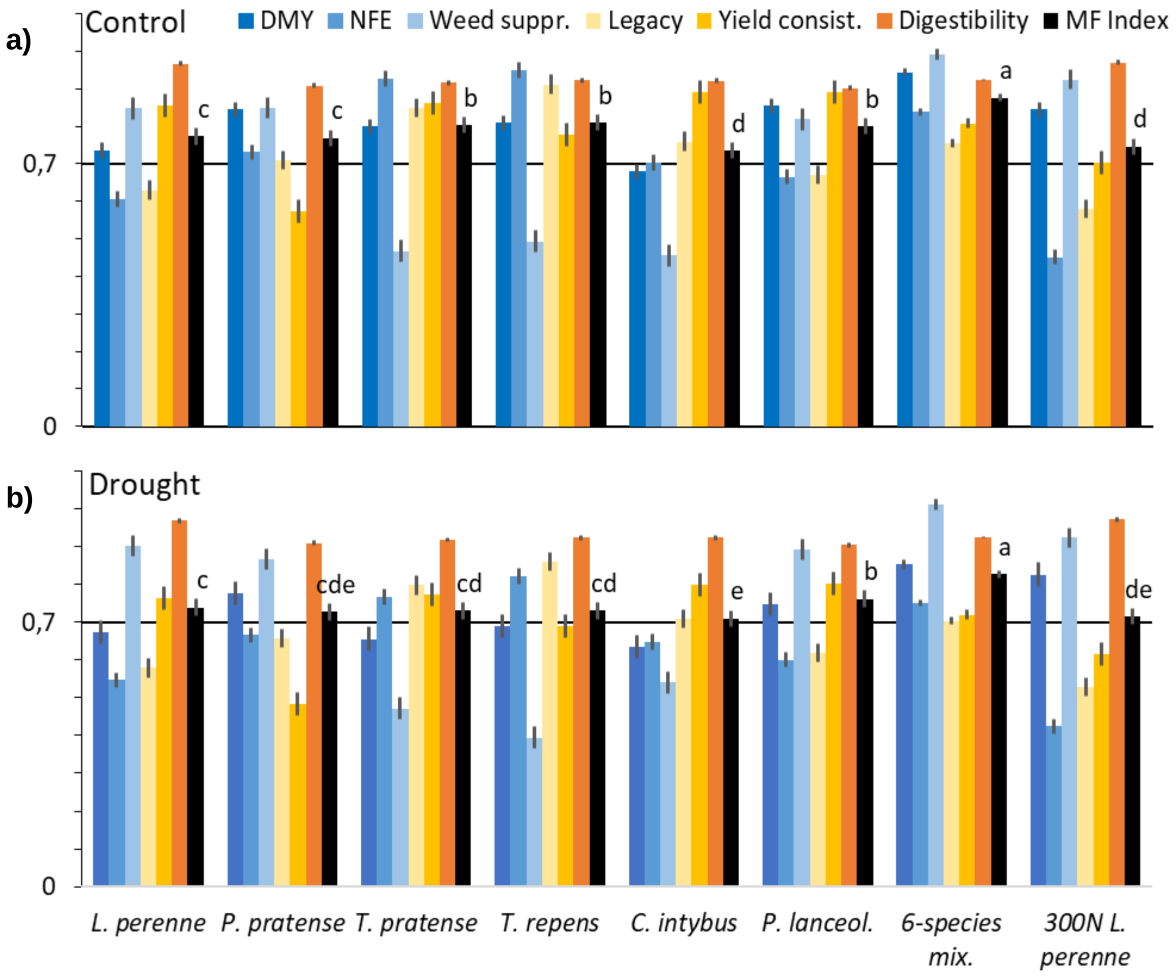

Figure 4.2. Modelled predictions for performances of selected communities across multiple functions, for (a) rainfed control and (b) drought conditions. Each bar represents the standardized prediction for the corresponding function for all 150N monocultures individually, the six-species equi-proportional mixture (centroid) and the 300N *L. perenne* monoculture. The black bar shows the MF index, calculated as the unweighted average across all functions. Error bars display the standard errors of prediction. Pairwise comparison tests were performed for the MF index (black bar) across the eight community types for rainfed and drought separately; within each group, bars that do not share a letter are significantly different (α = 0.05).

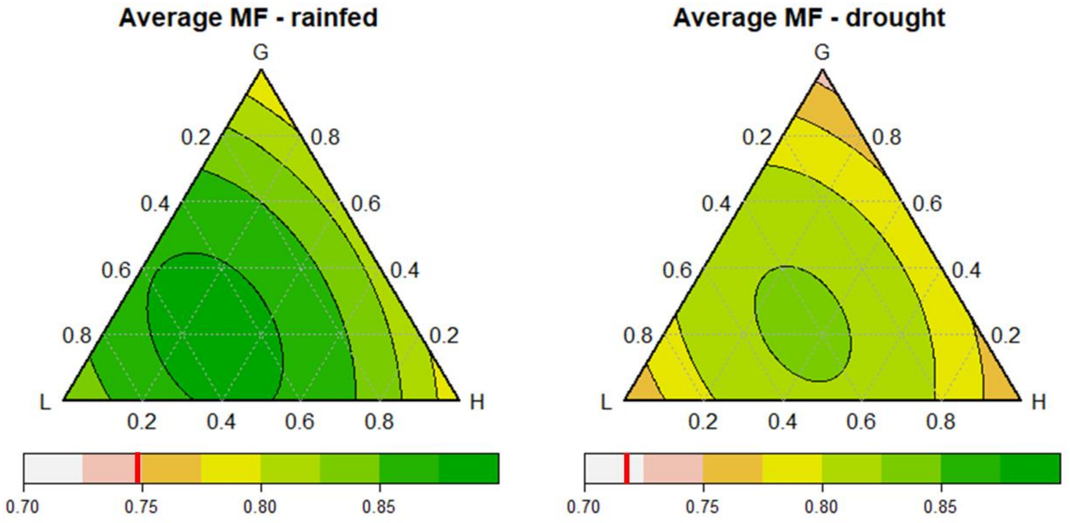

Figure 4.3. a) Contour plots showing the model predictions of the multifunctionality index (MF) from any mixture sown with grass, legume and/or herb under rainfed or drought conditions, assuming equal proportions for the two species from each FG. MF was predicted across the 3-dimensional functional group simplex space for two species (both from one functional group – the vertices in each ternary diagram), four species (with two species from each of two functional groups – the sides of each ternary diagram) or all six species (three functional groups – all interior points in the ternary diagrams). The red bar displays the prediction for the 300N *L. perenne* monoculture comparison.

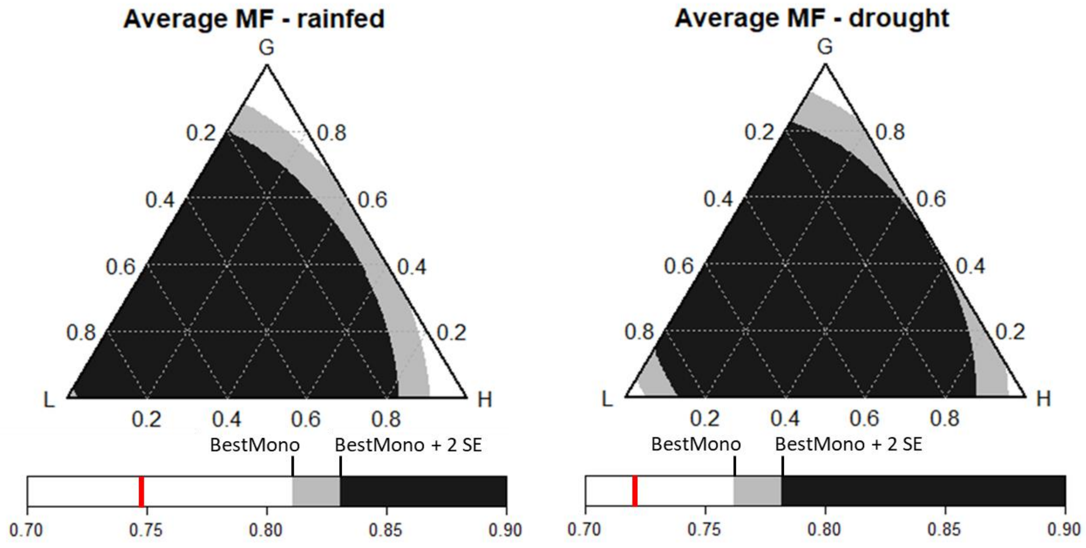

Figure 4.3. b) Comparison of the MF index of mixtures to the best performing monoculture (*T. repens* under rainfed and *P. lanceolata* under drought, displayed as “BestMono” in the figure) averaged across all functions. White area stands for when the predicted mixture MF is less than the best predicted monoculture (BestMono), grey area for mixture MF > BestMono, and black area for mixture MF > BestMono + 2 SE. Thus, the area in black displays where transgressive over-performance is significant in a conservative test. The red bar represents the 300N *L. perenne* monoculture performance.

Grassland multifunctionality increased with plant diversity. Indeed, to deepen the exploration of diversity effect on multifunctionality, we plotted the MF index across grassland mixtures in Fig. 4.3. The communities displayed in these ternary diagrams assume an equal proportion of each species in a FG (e.g. top vertex is 100% grass which is 50% *L. perenne* and 50% *P. pratense*). We noted that higher MF was achieved by more diverse communities (i.e. central area of the ternary diagram). In particular, communities sown with 20% to 80% legume delivered a higher MF index than the best monoculture under both rainfed and drought conditions (Fig. 4.3). In addition, we show that the 300N *L. perenne* monoculture MF index was below the range of performance across the predictions shown in the ternary diagrams (even the vertices which include only one FG, Fig. 4.3).

Interactions between functional groups enhanced multifunctionality

Three functions (yield consistency, legacy effect, and digestibility) were best estimated from species identities only (Table 4.1). For functions best described by a model including functional group interaction effects (DMY, NFE and weed suppression), the between-FG interactions (Grass*Legume, Grass*Herb and Legume*Herb) were always significant and positive (Table 4.1). Within-FG interactions (Grass*Grass, Legume*Legume and Herb*Herb) were generally smaller, but the significant ones were positive.

The MF index for mixtures was calculated as the weighted average of component species identity effects, to which interaction effects were added. Although only half of the functions show evidence of species interactions (Table 4.1), the overall effect (averaged across functions) was positive, as illustrated by the dome shape of MF index response in Figure 4.3,a. The magnitude of the benefit from species interaction for the MF index was strong enough to result in transgressive over-performance from a wider range of mixtures, i.e. mixtures outperforming the best monoculture (Fig. 4.3,b).

Discussion

We analysed the multifunctionality of an intensively managed grassland. We showed mixed outcomes on the effect of species identity and a manipulated water supply treatment, and a strong enhancement of ecosystem functioning due to plant interactions for some functions.

Diversity effect varied across individual ecosystem functions

Across the ecosystem functions studied, our results were consistent with literature from univariate analysis of diversity effect under similar management. Indeed, we found that strong between-FG interactions enhanced annual yield, nitrogen cycling and weed suppression, in line with previous work on four-species mixtures (Nyfeler *et al.*, 2009; Finn *et al.*, 2017; Connolly *et al.*, 2018). However, yield consistency only depended on species identity effects, with no evidence of interactions, which differs from Haughey *et al.* (2018) work showing enhanced consistency from four-species mixtures compared to monocultures. Similarly, we found no evidence of plant interaction effects on grassland legacy, in contrast with the legume * non-legume synergistic interaction found by Fox *et al.* (2020b) in the legacy effect of four-species grassland mixtures. Divergent results from literature about the effect of diversity on grassland digestibility prevent to conclude to a clear pattern. We found that digestibility was determined by species identities only, which seems to confirm results from Sanderson (2010a), suggesting that nutritive value is driven by functional composition. Due to the general lack of information on species identity effect of legume and herb species (e.g. nutritive value of material from monocultures), we cannot determine if enhanced digestibility in mixture found in literature can be allocated to diversity effect or to the added species effect (Roca-Fernandez *et al.*, 2016; Grace *et al.*, 2018).

Diversity effect on multifunctionality maintained under drought conditions

The benefit of diversity was relatively higher under drought than rainfed for DMY and NFE. For these functions, drought had a negative effect on species identities and a strong interaction effect that maintained under drought. This resulted in diversity effect (driven by interactions) being relatively higher under drought than rainfed (compared to the performance of the community). In a pot experiment investigating the effect of species richness and drought, Xu *et al.* (2022) showed that mixtures with higher diversity (seven versus four) better maintained the overyielding effect under drought conditions regarding aboveground biomass. However, they showed that the diversity induced resistance to drought was less obvious for other functions. Similarly, Hong *et al.* (2022) showed that biodiversity effect on ecosystem multifunctionality was stronger in stressful conditions, but that was not necessarily the case for belowground functions in grasslands stressed by drought. Our results are in line with these works, as diversity enhanced the mitigation of drought effect on productivity (e.g. DMY and NFE), but legacy effect being a soil related function was not affected by diversity neither under rainfed nor drought. It appeared that deep rooted herb species were relatively more competitive against weeds under drought conditions. On the opposite, the shallow-rooted *T. repens*, from which symbiotic N fixation might have been limited by the duration and strength of drought, was much less competitive against weeds under drought (Hofer *et al.*, 2017).

Our work shows strong between-FG interactions, resulting in a dome shape MF response that maintains under drought conditions compared to rainfed, despite species identity effects being lower (Fig. 4.3,a). Fry *et al.* (2018) found that the diversity-associated resistance to drought in multifunctionality was defined by plant traits. Similarly, Isbell *et al.* (2011) show that high diversity is required to maintain functioning across environmental changes. We illustrate such principles practically by showing that the equi-proportional six-species mixture maintained the magnitude of its benefit over the monoculture average even under drought (+0.095, Table 4.1). The equi-proportional six-species mixture also outperformed the best monoculture MF index by 0.066 under both rainfed and drought conditions (Table 4.1). Figure 4.3b shows that a wide range of diverse mixtures achieved a higher MF index than the best monoculture under drought. We note also that the 6 species equi-proportional mixture under drought outperformed or equalled all rainfed monocultures (Table 4.1).

Using a multi-model ensemble involving three different global climate models, Sweeney *et al.* (2008) predicted a reduction of 30-40% of summer rainfall in the South-East of Ireland by 2080, which corresponds to a reduction of approx. 75-100mm. The reduction in rainfall achieved by the rain shelters in this study was of same order of magnitude, with reductions of 122 and 98 mm for 2018 and 2019 respectively. The rainout shelters successfully reduced rainfall and soil moisture, but also have a combined effect on PAR, air temperature and humidity, wind speed etc. Although we refer to ‘a manipulation of water supply’, we acknowledge that the net contribution of combined changes in environmental factors will have caused the measured water supply treatment effects. Natural drought generally also goes hand in hand with variation in other changes in environmental factors, but the purpose of such study “was not to simulate a specific climate scenario, but to subject our system to severe stress to mimic extreme events that are predicted to become more frequent”, as in Hoekstra *et al.* (2015) who used a similar setup to investigate drought effects.

Functional groups ‘specialization’ results in trade-offs between species contribution to ecosystem functioning

As illustrated in Fig. 4.4, trends across functions show apparent FG ‘specialisation’, e.g. legume species performing well in N related functions (NFE, legacy effect) but not in weed suppression. Similar trends were found by Dooley *et al.* (2015). This specialisation can result in trade-offs among functions, as species performing high in some functions show poor performances in others (as indicated in Fig. 4.4, for instance, legume monoculture performs strongly in legacy effects but poorly in weed suppression, while herb monocultures perform better than legumes at suppressing weeds, but less well at legacy effects). Trade-offs between functions and fluctuations across years and growing conditions result in a larger number of species required to achieve a larger number of ecosystem functions (Gamfeldt *et al.*, 2008; Zavaleta *et al.*, 2010). Trade-offs are also illustrated by the crossing of lines in Fig. 4.4. If focusing on monocultures, choosing a single FG for its performance for a given function might restrict the potential to achieve high performance in other functions. Digestibility was quite similar from across functional groups, thus not associated with particular trade-offs. Comparing performances under rainfed and drought conditions (Fig. 4.4), we can note similar patterns regarding trade-offs occurrence, as the global trend is a reduction in performances under drought.

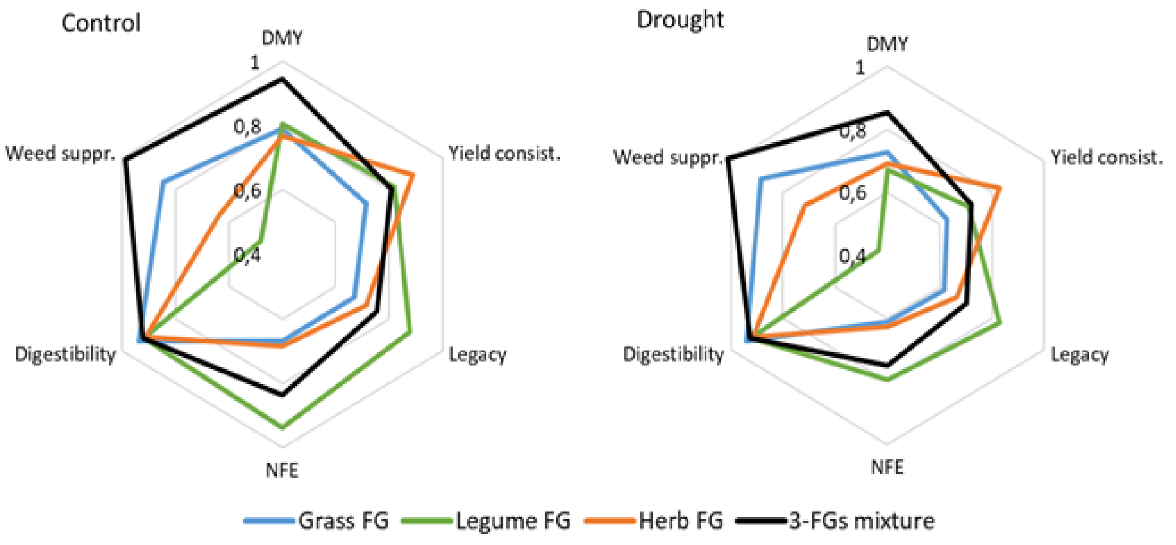

Figure 4.4. Radar chart displaying predicted standardized performance (on a scale from 0 to 1) across each function for the average of the monocultures of species from each functional group. The performance of the balanced mixture of the 6-species from the three functional groups is also displayed (black line). Both rainfed control and drought treatment are represented.

When mixing species, the divergent patterns from different species could prevent mixtures overyielding (Slade *et al.*, 2019). This was the case across functions where no interaction effect were found (Legacy effect, consistency and digestibility). However, strong positive interaction effects in other functions helped to compensate, and allow the occurrence of overyielding (Fig 4.3,b). Similar results were found by Suter *et al.* (2021) who did not find trade-offs associated with synergistic interactions between functions. They also found a strong benefit of diversity with four species mixtures overall performance reaching up to 1.9 times that of the average monoculture.

By disentangling specific identity and interaction effects in ecosystem functioning, the Diversity-Interactions approach helps to select the most efficient combinations in designing multifunctional communities. This is a great advance beyond species richness methods (Byrnes *et al.*, 2014; Gamfeldt and Roger, 2017), for practical application of ecological principles to intensively managed grasslands. Focusing on species with high complementarity opens the possibility to overcome the requirement for targeting higher richness to achieve multifunctionality.

Species interactions underpin overall transgressive over-performance

We show that a wide range of mixtures outperformed the best monoculture MF index (Fig. 4.3,b). This transgressive over-performance is achieved due to synergistic interactions between grassland species, especially across functional groups. However, only three functions out of our six showed evidence of interaction effects (DMY, NFE and weed suppression). No overyielding was identified for legacy effect, yield consistency, nor digestibility. In addition, species interactions do not ensure transgressive over-yielding for a given function, as it requires that the interaction effects have higher magnitude than the difference between the mixture’s species identities and the best monoculture (see Fig. 2.1 in Chapter 2). Transgressive over-yielding is also due to species performing quite similarly (i.e. small differences between identity effects when averaged across functions, Finn *et al.* (2013))

A critical point is that mixtures of two species from the same FG (vertices in Fig. 4.3) never significantly outperformed the best monoculture (insufficiently strong within-functional group interactions). However, all mixtures of four species from two FG did achieve transgressive over-performance, as long as legume species were involved and that one FG did not represent >80% of the mixture. This assumption is true under both rainfed and drought conditions, and shows the benefit of balanced FG-rich grassland for high ecosystem functioning. It also reminds the importance of considering species composition and proportions rather than species richness. Such balanced communities correspond to the central area of the simplex design (shown in the ternary diagram, Fig. 4.3), where the dome shape of the MF index response shows the robustness of high levels of ecosystem functioning across varying species proportions.

To assess the effect of species interactions, Suter *et al.* (2021) used the log response ratio described in Hedges *et al.* (1999). By averaging the natural logarithm of the ratios between mixtures performance and the corresponding weighted monoculture average across functions, they showed that interactions between legume and non-legume species were the drivers of overyielding across functions. Here, we extend this conclusion to any interactions among grass, legume and herb FG. Moreover, we show that the diversity effect is sufficiently robust for a wide range of mixtures to out-perform any monoculture from the species pool, across contrasting environmental conditions.

Multifunctional approach highlights limitations associated with low-diversity, high-input management

By including functions related to agronomic and environmental characteristics of grasslands in a combined analysis, we draw conclusions on a more comprehensive effect of farming practices.

We included the 300N *L. perenne* monoculture comparison in the design as it represents a very common management of grassland in temperate climates (Dillon *et al.*, 2020). It is supposed to fulfil intensive farms objectives, *per se* productive and persistent grassland with good feeding value. Indeed, the 300N *L. perenne* is among the best-performing monoculture communities for dry matter yield, digestibility and weed suppression, as expected. However, environmental, economic and climatic concerns are rising, and require a greater incorporation of key sustainability metrics. Alongside yield, grasslands are expected to deliver a wide range of ecosystem services, including i) efficient use of fertiliser to reduce water and air pollution (Steenvoorden *et al.*, 1986; Abalos *et al.*, 2021; Cummins *et al.*, 2021; Mahmud *et al.*, 2021)); ii) resilience to climate variability to stabilise production (Haughey *et al.*, 2018; Coronese *et al.*, 2019; Hossain and Li, 2020; Emadodin *et al.*, 2021) and iii) increased soil fertility to enhance carbon sequestration and avoid soil degradation (Houghton *et al.*, 2001; Lemaire *et al.*, 2015; Carozzi *et al.*, 2021).

We measured three ecosystem functions involved in fertiliser efficiency (NFE), resilience (yield consistency), and soil fertility (legacy effect). For these three functions, 300N *L. perenne* was the lowest performing monoculture. We conclude that low-diversity, high-input management result in a trade-off between agronomic performance and functions associated with environmental performance, thus questioning the relevance of such management for achieving sustainable agriculture. On the other hand, focusing on the six-species equi-proportional mixture as a high-diversity, low-input management, we note that the high performances in dry matter yield and weed suppression are not associated with trade-offs in NFE or consistency. Thus, plant diversity appears as a practical way to avoid trade-offs between agronomic performance and sustainability of temperate grasslands.

Conclusion

Using a multivariate approach, we show that plant diversity strongly enhanced ecosystem functioning in intensively managed grasslands. Multifunctionality was particularly promoted by balanced mixtures of grass, legume and herb species that outperformed the best monocultures performances under contrasted weather conditions. Finally we show that an increase in plant diversity is much more efficient than an increase in fertiliser use in stimulating grassland multifunctionality.

Supplementary material

Table S4.1. Variance-covariance matrix as estimated from the model described in eqn. 4.2. Fixed parameter estimates are in Table 4.1. An unstructured covariance structure is assumed for multivariate ecosystem functions from the same subplot, while a covariance is induced on individual ecosystem functions from two subplots within the same main plot to respect the split-plot nature.

			Main plot											
			Subplot 1						**Subplot 2**					
			DMY	**Digest.**	**Legacy**	**NFE**	**Yield consist.**	**Weed suppr.**	**DMY**	**Digest.**	**Legacy**	**NFE**	**Yield consist.**	**Weed suppr.**
Main plot	**Subplot 1**	**DMY**	0.0016	-0.0001	-0.0002	0.0007	-0.0016	0.0000	0.0000	0	0	0	0	0
		Digest.	-0.0001	0.0002	0.0000	0.0000	-0.0001	0.0000	0	0.0001	0	0	0	0
		Legacy	-0.0002	0.0000	0.0044	0.0021	0.0005	-0.0001	0	0	0.0002	0	0	0
		NFE	0.0007	0.0000	0.0021	0.0020	-0.0003	-0.0001	0	0	0	0.0001	0	0
		Yield consist.	-0.0016	-0.0001	0.0005	-0.0003	0.0070	0.0000	0	0	0	0	0.0000	0
		Weed suppr.	0.0000	0.0000	-0.0001	-0.0001	0.0000	0.0027	0	0	0	0	0	0.0011
	Subplot 2	**DMY**	0.0000	0	0	0	0	0	0.0016	-0.0001	-0.0002	0.0007	-0.0016	0.0000
		Digest.	0	0.0001	0	0	0	0	-0.0001	0.0002	0.0000	0.0000	-0.0001	0.0000
		Legacy	0	0	0.0002	0	0	0	-0.0002	0.0000	0.0044	0.0021	0.0005	-0.0001
		NFE	0	0	0	0.0001	0	0	0.0007	0.0000	0.0021	0.0020	-0.0003	-0.0001
		Yield consist.	0	0	0	0	0.0000	0	-0.0016	-0.0001	0.0005	-0.0003	0.0070	0.0000
		Weed suppr.	0	0	0	0	0	0.0011	0.0000	0.0000	-0.0001	-0.0001	0.0000	0.0027

Chapter 5

Discussion

Thesis overview and original contribution to knowledge

I investigated whether diversity enhanced ecosystem functioning in intensively managed grassland and the extent to which an experimental severe weather event modified diversity effects. This work contributes to general knowledge of the BEF relationship through better understanding of species identity and interaction effects in agro-ecosystems, in a changing environment.

Chapter 1 - The first chapter of the thesis introduced ecological principles about plant diversity and interactions in grasslands, and their effect on ecosystem functioning in natural conditions. I then explored the transferability of such principles to intensively managed grassland systems. Finally, I discussed the implications in terms of experimental design and analysis for investigating the biodiversity and ecosystem functioning relationship (BEF) in intensively managed grasslands exposed to environmental stress.

Chapter 2 - This work is based on an experiment that manipulated plant diversity in intensively managed grassland. Communities of one to six species (and one to three FGs) were studied through measurements of their yield outputs and under two water supply treatments to simulate the effect of a weather disturbance. I showed that increased diversity can be more efficient than increased fertiliser use in stimulating yield (comparing outperformance of mixtures over monocultures at 150 kg N ha^{-1} yr^{-1} to fertiliser effect from 150 to 300 kg N ha^{-1} yr^{-1} in a *Lolium perenne* monoculture). Interactions between legume species and either grass or herb species were the main driver of overyielding in mixtures, although grass * herb interactions were also significantly positive. For example, the equi-proportional six-species sward achieved higher total annual yields than those from the average of the six component monocultures under both rainfed (+ 21%) and drought (+25%) conditions. Transgressive overyielding occurred across a wide range of mixtures. The six-species mixture under drought outperformed the rainfed average monoculture and the 300N *L. perenne* monoculture. I concluded that a positive BEF relationship enhanced grassland yield and mitigated drought effects under intensive management.

Chapter 3 - In the third chapter, I investigated the relationship between plant diversity and grassland legacy effect, *per se* the net residual effect of grassland diversity on a the yield of a follow-on model crop in a rotation. Legume species were the best in stimulating follow-on crop yield, with crop yield being 27% higher when following a legume monoculture than a non-legume one. I found no evidence of species interactions for this function; thus, multi-species swards performed the same as the weighted average of component species, and the legacy effect increased proportionally to the legume proportion in the grassland phase. Drought reduced the legacy effect uniformly across plant communities. Additional fertiliser applied to grass monoculture did not enhance follow-on crop yield and the 300N *L. perenne* monoculture had the lowest legacy effect in the experiment. The legacy effect from the high-diversity, low-input community was of average magnitude, but in combination with its high yield in grassland phase, it outperformed the 300N comparison in both phases. The relative importance of grassland and crop phase would influence recommendations about grassland sown community. I show that a diverse sward with 33-75% sown legume proportion would perform well across the whole rotation.

Chapter 4 - After having investigated the BEF relationship for two functions separately (annual harvested biomass and legacy effect), I performed a multifunctional analysis to assess the effect of increased plant diversity on multiple agro-environmental performances of a grassland community. I found that plant diversity was strongly associated with higher multifunctionality (MF). Averaged across functions, the equi-proportional six-species sward under drought condition outperformed or equalled any rainfed monoculture. Overall, the diversity effect increased the MF index by 12%, comparing the six-species equi-proportional mixture to average monoculture. In contrast to increased diversity, a higher fertiliser use did not enhance multifunctionality, as the MF index for *L. perenne* decreased by 3% when fertiliser input was doubled from 150N to 300N. The 150N *L. perenne* monoculture already having a low MF, and the fertiliser increase having an additional negative effect on fertiliser efficiency, yield consistency and legacy effect, resulted in the 300N *L. perenne* monoculture to show one of the lowest values of MF index among the studied treatments. Drought reduced multifunctionality by lowering the identity effects across most functions. Plant interactions were not affected by drought and thus acted as a way to mitigate drought effect. Multi-species swards combining grass, legume and herb species are a practical tool for ecological intensification and drought mitigation of agro-ecosystems, for a range of functions extending beyond yield alone.

The original contribution to knowledge of this thesis can be summarized through the following points:

- For annual yield, weed suppression and nitrogen fertiliser efficiency, a positive BEF relationship led to transgressive overyielding via strong synergistic species interactions. The effect of interactions between legume and non-legume species were stronger than within-FG or grass * herb interaction effects. I identified the potential of herb species for inclusion in intensively managed grasslands, as they interacted strongly with legume species and their identity effects were quite similar to other species.

- For grassland annual yield, the occurrence of a two-month summer drought had a negative impact on the identity effects of most species. Legumes were more drought sensitive than other FG and the herb *C. intybus* was the most resistant. Since drought did not impact species interactions, the absolute magnitude of benefit on annual yield due to interspecific interactions was maintained under drought. Thus, the relative contribution to mixture yield due to species interactions was higher under drought conditions. To illustrate the benefit of plant diversity, I identified the wide range of mixture compositions that achieved transgressive overyielding across the design space.
- The design of grassland leys in rotations is very important, and can have a very strong influence on the magnitude of the legacy effect. No evidence of an effect of species interactions was found in the legacy effect of the grassland ley on a follow-on crop. Thus, no overyielding appeared for this ecosystem function as the legacy effect was solely driven by the species identities of the preceding grassland. Legume species delivered the highest legacy effect. In a grassland-crop rotation, a legume-rich diverse grassland would balance high performances for each phase. The poor legacy effect from the high input, low-diversity comparison showed strong evidence that a high nitrogen *L. perenne* monoculture is not a desirable option for enhancing productivity in rotations.
- Investigating the effect of intensive grassland on multifunctionality, I found that the BEF relationship depended on the function studied: interaction effects were not evident for yield consistency, digestibility and legacy effect. However, positive interactions in other functions (weed suppression, nitrogen fertiliser efficiency and annual yield) were sufficiently strong to enable transgressive over-performance of more diverse multispecies mixtures (averaged across all functions). This transgressive over-performance was achieved by any mixture across both rainfed and drought conditions, as long as the legume proportion comprised between 20% and 80%. Finally, trade-offs between functions appeared in monocultures, but was partly alleviated in mixtures, due to positive interaction effects on some functions.

Implications for intensively managed agro-ecosystems

Species selection for intensively managed grasslands

In intensively managed agro-ecosystems, plant communities are designed by sowing species selected and combined to perform in targeted functions. This thesis brings better knowledge on the effect of varying plant composition, under either normal or stressful weather conditions, in order to help recommendations about sown communities.

Productivity is the main targeted function in intensively managed systems, where the highest yielding species are selected, and often sown as monocultures. This results generally in species pool with acquisitive traits, for which management is adapted with regular nutrient (fertilisation) or water supply (irrigation). However, concerns about these practices and changing environment bring interest to consider a wider range of ecosystem functions in the selection of plant species. In addition, I show in Chapter 2 that diverse plant communities can yield more than the best performing monoculture under intensive management. A restricted species pool focused on acquisitive traits could compete with the functional distance between species, which was found to be correlated with better ecosystem functioning (Cadotte, 2013). On the opposite, a very large species pool would comply with large functional diversity, but with a higher risk that some species would not persist to a sufficiently high abundance to have a significant contribution to outcomes. After decades of intensive management of species rich grasslands, Roscher *et al.* (2007) noted a strong reduction in plant diversity and thus had to balance a 60 species pool diversity experiment in semi-natural grassland with a selected nine species pool trial for intensive management. Grace *et al.* (2018) found species disappearance in an intensively managed nine-species sward that did not perform better than a six-species sward, in the same conditions. The scope of the BEF relationship in intensively managed grasslands relies thus within the pool of species adapted to intensive management and fulfilling high performance in the functions of interest for farmers.

An *a priori* selection of species pool from the highest performing species for a given function could be detrimental to high performance in other functions. The multi-functional approach used in this study showed that the BEF relationship was strongly dependent on the function studied, as interspecific interaction effects only occurred in some cases. When no interaction effects are involved, monocultures can appear as the best choice (e.g. Legacy effect, Chapter 2), but the chances that this species will be the best performing decreases with the number of functions studied. The multivariate approach in Chapter 4 also highlighted trade-offs between functions for monocultures, i.e. species grown in monoculture could not perform well across all functions. Schulze *et al.* (2018) point that relative advantage of diverse communities observed on the productivity of natural ecosystems can be cancelled by intelligent management of single high-potential species in intensively managed systems. However, they recognize that diversity brings benefits when considering stabilizing strategies to face climate variability.

If building a species pool based on performance for one single function, we potentially 1) give up on higher performances in other functions and 2) miss strong interaction effects by narrowing functional diversity. The first point can be tackled by creating indices gathering practical indicators under a predefined management (e.g. the Pasture Profit Index, O'Donovan *et al.*, 2017), but consideration of environmental aspects, wider range of management and adaptability to mixtures remains poor. About the second point, Litrico and Violle (2015) remind that breeding strategies are not yet adapted to select genotypes for the building of genetically heterogeneous communities. Indeed, the occurrence of transgressive overyielding points that mixture outreach the potential of single genotypes, thanks to synergistic interactions. Thus, a selection based on interaction potential could be more appropriate than a selection based on performance in monocultures. Although not intuitive, this could result in some species with lower performance on a key function (e.g. yield), to be relevant for inclusion in a mixture. This was the case in the present study, where the inclusion of species from a novel functional group (herb) proved to enhance the performance of mixtures. Indeed, these species were not commonly grown as monocultures because did not show the highest yield potential, but due to strong interactions with other FG, they can play an important role in enhancing BEF relationship. In an intensively managed experiment studying the inclusion of herb species in a grass – clover ley, Cong *et al.* (2018) showed that although in most cases the herb monocultures were performing lower than the grass – clover ley, mixtures of all three FG could outyield the grass – clover one. In parallel, they showed a decreasing proportion of herb over a short period of time, attesting to a low adaptation to intensive management. Despite these unfavourable characteristics (adaptability and performance as monoculture), herb species still showed a potential to enhance weed suppression and biomass yield.

Species selection for intensively managed grasslands needs a more holistic approach than the assessment of the performance of single species (monocultures) to single functions (e.g. yield). Indeed, species pool used for intensively managed grasslands must answer good adaptation to management and high potential for the purposed functions, but most importantly, high interaction potential with other selected species is crucial to enhance strong BEF relationship. This work shows that communities with three FG (grass, legume and herb) had high chances to outperform the best monoculture. However, the occurrence of transgressive overyielding depended on the relative abundance of FG and the ecosystem function studied.

Combining selected species to optimize grassland performances can result in transgressive over-performance

Once the species pool is defined, based on individual potential, the sown proportions must be decided ahead of establishment, to maximize the delivery of ecosystem services of interest. To recommend the best species combination for enhanced BEF relationship in intensive agro-ecosystems, a precise assessment of plant interactions is essential for the function of interest. I showed that plant interactions can vary a lot across functions, resulting in different optimal combinations of species depending functions. Thus, a multifunctional approach with precise definition of diversity is required for the assessment of the best species combination. Eisenhauer *et al.* (2016) discussed the links between real world ecosystems and experimentation, confirming that a translation of ecological of the BEF relationship principles from natural ecosystems to intensive agro-ecosystems should be accompanied by adjustments in method, especially in the characterisation of biodiversity.

The Diversity-Interactions approach used in the present study, enables to describe diversity effect more precisely, through species identity and pairwise interaction parameters (Kirwan *et al.*, 2009). As suspected from semi-natural grasslands (Brophy *et al.*, 2017a), this quantification pointed dissimilarities in the strength of interactions (e.g. legume-herb > grass-herb interaction). Differences amongst interaction effects also shows the limitations of the evenness for a detailed understanding of interactions and for more precisions in predicting peak performances. Indeed, relating diversity effect to the evenness of species relative abundance is useful for determining the occurrence of a global interaction effect (Kirwan *et al.*, 2007), but I highlight in Fig. 1.3 that this approach can be misleading in case of dissimilarities among interactions. Species sown proportions are thus to be determined from both their identity effects and the sum of all interaction effects.

A relevant choice of the species proportions can result in the occurrence of transgressive over-performance, meaning that a mixtures outperforms the best monoculture (as shown in Chapter 2 and Chapter 4). Two major aspects are pointed to greatly increase the chances of transgressive over-performance; 1) that species identity effects are not extremely dissimilar; and 2) that strong interactions between species overcome the difference between the weighted average of the species, and the best-performing monoculture (Nyfeler *et al.*, 2009). Still, the fact that transgressive over-performance was identified on a wide range of mixtures highlights the robustness of plant diversity benefit on annual yield under intensive management (Chapter 2). This robustness was confirmed in Chapter 4, where half of the functions did not show evidence of interactions between species or FG, but transgressive over performance was still achieved from a wide range of communities.

Plant diversity mitigates extreme weather events

The occurrence of an extreme summer drought did not affect plant interactions, despite affecting identity effect. Thus, communities with higher diversity were impacted by drought at the same magnitude as lower diversity ones. Due to generally positive interaction effects, diverse communities were then relatively less impacted by drought. Moreover, I show that annual yield (in Chapter 2) and multifunctionality (in Chapter 4) of some mixtures under drought can outperform most monocultures under rainfed conditions. The consequences may be crucial for farming systems and insurance companies, if larger scale results confirm that plant diversity ensures a higher performance than the best monoculture, independently of the occurrence of an extreme summer drought.

In this thesis, I also show that drought impact on species identity and interaction effects might result in a shifts in the species proportions for the best performing mixtures in the occurrence of drought, for some functions. However, these shifts were minor and globally, balanced mixtures of grass, legume and herb were among the best performing communities for DMY, NFE, weed suppression and MF index. This robustness was due to interactions maintaining, while identity effects were affected by drought. Such results differ from the conclusions of Grant *et al.* (2014) who found a shift in the type of interactions when a disturbance occurred. The authors state that shifts in the BEF relationship are linked to plant composition. However, across six functions studied for quite a diversity of plant composition, I found no evidence of drought induced shifts in interactions.

The results presented in this thesis focus on annual scale at least, in contrast to several works assessing the effect of drought that investigated separated periods before, during and after drought (Britaňák *et al.*, 2010; Vogel *et al.*, 2012; Hofer *et al.*, 2016; Haughey *et al.*, 2018). The seasonal approach is of great importance for the assessment of resistance during the disturbance and magnitude and speed of the recovery (resilience). On the opposite, considering annual results instead, I measure the overall net effect of resistance and recovery. This allowed easier translation to farm-scale indicators (e.g. effect on annual yield). Despite the different scales, results from this thesis can be crossed with results from seasonal assessment. Similarly to Hofer *et al.* (2016), I found quite a minor effect of drought on overall growing season, and the diversity associated mitigation of drought agree with results from Haughey *et al.* (2018). However, Vogel *et al.* (2012) found an increased sensitivity to drought with lower diversity and higher intensity management where I did not find a higher impact of drought on the low-diversity, high-input comparison.

The present study assessed the residual effect of drought on the follow-on step of a rotation, which is novel in intensively managed plots. The findings about the potential effect of drought even after reseeding warns about shorter term conclusions. The effect of drought during grassland phase was influenced by plant diversity and input level, similarly to Vogel *et al.* (2012) conclusions, but the residual effect of drought on the follow-on crop (legacy effect) was constant across all treatments. I speculate that this constant effect happens either because 1) legacy effect of drought depends on soil processes that are independent to former plant cover or management, or 2) that the effect was too small and/or variable to determine links between variations and treatments. An experimental design to measure such effect is introduced in the future work section.

Divergent results about the effect of drought between the study presented and results from the literature were identified about few aspects and could be explained by site-to-site variation in drought effects. Indeed, soil properties can have a strong influence of the severity of drought (Buttler *et al.*, 2019). Simulating drought following the same protocol in two sites, Haughey *et al.* (2018) achieved different levels of stress, resulting in dissimilar effects on plant communities. A larger scale exploration of the effect of drought on plant communities is of great importance in a context of climate change. However, better knowledge about this aspect can only be sought via multi-site experiments.

Comparative benefits of increased plant diversity over increased application of nitrogen fertiliser

Compared to increased fertiliser use, an increase in plant diversity seems more efficient to promote ecosystem functioning and prevent environmental and economic cost. Indeed, I show in Chapter 2 that the net effect of plant diversity can outperform the extra yield gained by increasing fertiliser use. In Chapter 3, I point that the extra nitrogen applied did not result in a higher follow-on crop yield, and thus might have been lost from the system. Finally, multi-variate analysis in Chapter 4 unveils that a higher rate of fertiliser does not enhance grassland multifunctionality, while higher plant diversity strongly promotes it.

In case of a summer drought, the high-input, low-diversity comparison showed a good resistance to drought across functions (Table 4.1), however it was still outperformed by most mixtures (Fig 4.3). Van Sundert *et al.* (2021) investigated the effect of fertiliser combined with the effect of drought on grassland productivity, and showed that nutrient addition increased grassland sensitivity to drought. They also found that grass species were more affected by drought than legumes and forbs, and conclude that grass-dominated fertilized swards are not adapted to the increased weather disturbance due to climate change. The results presented in Table 4.1, differ from those in Van Sundert *et al.* (2021), as the 300N grass monoculture was generally less impacted by drought than other communities. However, the fact that grassland mixtures outperformed the high input grass monoculture was a constant across this thesis.

The high fertiliser comparison was often among the lowest performing communities across functions (Chapters 2, 3 and 4), with increased inputs not resulting in over-performance for most functions (except annual yield and weed suppression). Investigating the effect of fertiliser input (150N *versus* none), Šidlauskaitė *et al.* (2022) found that the yield of grass monocultures was enhanced by fertiliser; but this effect was very variable across mixtures of 60% grass and 40% legumes, varying species compositions. The lack of efficiency of this off-farm input adds to the questioning about the economic cost and environmental impact associated with fertiliser (Ravishankara *et al.*, 2009; Leip *et al.*, 2015; Mahmud *et al.*, 2021). I show that contrarily to grass dominated swards receiving high rates of fertiliser, plant diversity can enhance farm agro-environmental performance with low use of inputs. I thus introduce practical ways to answer European Union Farm to Fork strategy that plans to reduce fertiliser use by 20% before 2030 (European Commission, 2020). This is also a step forward in addressing the United Nations sustainable development goals (UN and Desa, 2016) for which Coscieme *et al.* (2021) precisely identified a lack of references combining biodiversity and climate goals.

Limitations of the work and knowledge gaps remaining

What about higher diversity?

The effect of diversity beyond six-species remains unclear for intensively managed grasslands (Fig. 5.1). This study suggests higher ecosystem functioning from six-species mixtures than the most commonly studied four-species mixtures. However, several contrasting scenarios could be considered about the BEF relationship at higher diversity. Recalling the work from Cadotte (2013) about stronger advantage from functionally distant species, higher diversity could result in functional redundancy and thus to a plateau. This saturation effect has been pointed in most studies about intensively managed systems going beyond four species, with no improvement in yield beyond three or four species (Roscher *et al.*, 2007; Sanderson, 2010b; Grace *et al.*, 2018; Lorenz *et al.*, 2020; Moloney *et al.*, 2020). Counterexamples show that larger species pool can result in the inclusion of high performing species that could then fit in lower diversity mixtures (Jing *et al.*, 2017), but a larger species pool is also likely to include lower performing species and thus result in a negative slope in the BEF relationship beyond a peak (Fig. 5.1). Most studies on BEF relationship only investigated the effect of species richness or compare communities through a categorical approach (e.g. ANOVA). We could imagine that the exploration of other dimensions of diversity would result in the identification of communities with lower diversity reaching peak performance (Fig. 1.3). On the opposite, considering a larger panel of grassland outcomes or environmental conditions could justify the inclusion of extra species (Isbell *et al.*, 2011; Isbell *et al.*, 2017).

As inclusion of higher diversity can be costly to agro-ecosystem managers, economic studies will need deeper information about the potential inflection point in the BEF relationship (Fig. 5.1, Finger and Buchmann, 2015; Schaub *et al.*, 2020). On the opposite, works from Prieto *et al.* (2015) and Meilhac *et al.* (2019) showed that within-species diversity (i.e. mixing cultivars) could bring higher grassland productivity and stability. Mixing selected cultivars is a specificity of intensively managed agro-ecosystems and these works highlight how the BEF relationship could be promoted by designed inclusion of this extra facet of diversity. More research is needed to investigate the relative benefit of within-species versus across species diversity, as well as potential overlaps of these two diversity effects.

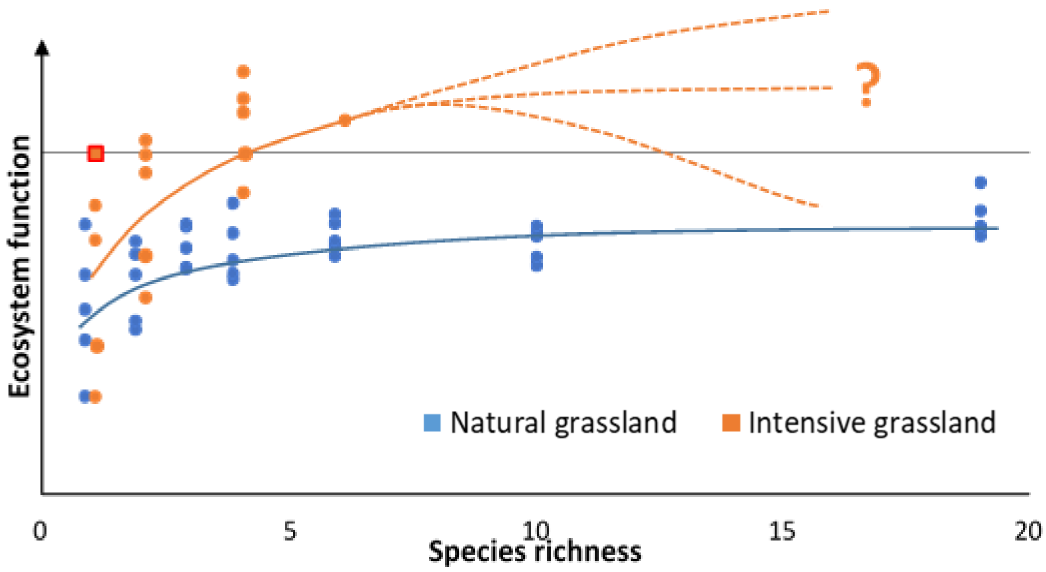

Figure 5.1. Conceptual illustration of the BEF relationship across species richness in natural grassland (in blue) as described in the literature, and in intensively managed grasslands (orange) as developed in the present study. By comparing species grown on their own, agricultural research selected the most adapted species to the ecosystem function of interest under intensive management (red square). The work presented in this thesis highlights that multi-species mixtures designed to favour plant complementarity can deliver higher outcomes than the best monoculture performance (black line). Dotted orange curves remind that further plant diversity remains to be explored under intensive management to unveil potential increase in overyielding (top curve), saturation effect (medium curve), or inversion of the BEF relationship (lower curve).

Achieving similar outcomes with lower diversity

How much diversity is enough to reach the highest performance? In intensively managed agro-ecosystems, the highest performing community is sought, as it can be established and managed to maximize its potential. Contrarily to natural and semi-natural grasslands, the interest is thus not on the slope of the ecosystem function along richness levels (Fig 5.1), but on the single best community. Following conventional selection, the best monoculture is often used in intensively managed grasslands (point highlighted in red in Fig. 5.1). I show in this thesis that diverse communities can outperform the best monoculture. However, a positive diversity effect on performance does not implies that a community with higher diversity would outperform a lower diversity one (see top point in Fig. 5.1 being reached at species richness = 4).

To characterize the community delivering the best performance, a precise knowledge of species identities and interactions is needed. Top performance can be achieved with quite low diversity, e.g. a four-species mixtures receiving 50N can outperform a grass-only mixture receiving 450N (Nyfeler *et al.*, 2009) , but this requires combining species at the right proportions. The principle of targeting functional distance to enhance synergistic interactions suggests that within-FG interaction effects would be smaller than between-FG interaction effects. Indeed, I found stronger between-FG interaction effects than within-FG interaction effects. However, some within-FG interactions were also significantly positive. Aiming for BEF relationship optimization, it would be interesting to assess if each FG would be better represented by the best performing species of that FG or by a mixture of species from that FG, in a larger assemblage.

One possibility would be that combining the best legume, grass and herb species (a three-species mixture) would deliver higher outputs than a six-species mixture. The choice of mixtures composition and proportions would then be determined by communities with smaller diversity outperforming the higher diversity ones. The present design is adapted to an assessment of interactions within and across FG, instead of interactions between each pair of species (Fig. 5.2). This could result in a loss in information in case the two species from a FG interacts differently with species from another FG. Without knowing the interaction effects of all pairs of species, the assessment for a reduction in plant diversity that would not lower performance is limited. A within-FG transgressive overyielding test could inform if it is preferable to keep one or more species in a given FG, i.e. a test among communities built from species from on FG, to conclude whether a mixture outperforms the best species from this FG. If no mixture outperform the best monoculture, one species would be sufficient to achieve at least similar performance than any mixture from this FG. The limitation arise when mixing with other FG and refers to the unreasonable assumption that all species from a FG interact similarly with other FG (Fig. 5.2).

An ecological perspective of this question was brought in the functional redundancy described by Kirwan *et al.* (2009). Indeed, the test for functional redundancy aims to conclude whether two species or more can be considered as behaving the same, i.e. that they do not differ in their contribution to a community outcome. This requires the following conditions (highlighted in Fig. 5.2,a): species have to be similar in their identity effect and interaction effect with other species, and they cannot interact with each other (Kirwan *et al.*, 2009). I bring here an agronomical perspective, as the assessment proposed for diversity optimization aims to test if a reduction of one species in the pool would challenge the delivery of the highest outcome possible. The conditions for selecting one single species from a FG would then be based on the fact that the other species from this FG are not part of the best performing community for the ecosystem functions of interest. To achieve such assessment, the knowledge of all species pairwise interactions is required. The information delivered by the design used in this study does not allow to assess the interaction effect of each species (Fig 5.2,b). Such interactions are illustrated in Fig. 5.2,c. In the particular case displayed, species A1 outperforms species A2, but stronger interactions with other FG lead to species A2 being the best species to be included in a mixture for this ecosystem function. It is however unlikely that one single species would be the best option from a given FG across functions or varying environmental factors (Isbell *et al.*, 2011). Indeed, I showed in Table 4.1 that no species outperformed the other one from the same FG across all functions and treatments.

Refining our understanding of species interactions

The experiment was designed with a focus on between-FG interactions, and the design did not include two-species mixtures from two different FG (i.e. no mixture with one legume and one herb species, one grass with one herb, nor one grass with one legume). This grouping of interactions at FG scale limited the assessment of species pairwise interactions. Indeed, one species from a FG could interact stronger than the other one from the same FG with species from other FGs. The information lost could be minor for FG with functional redundancy, but this may not be a reasonable assumption for FGs with wider diversity (i.e. larger variations within functional traits), like herb, recalling that interactions are enhanced by functional distance (Díaz and Cabido, 2001; Cadotte, 2013). In intensive agro-ecosystems, the reasonable size of the species pool (generally <10) should motivate the assessment of interactions to the species pair level.

Another key aspect about interactions is that DI models as used in this work supposed a symmetric interaction term with a parabolic dome. In other terms, the interactions between species is assumed to be driven by the proportion of each species involved in the mixed, without extra weighing. Taking the example of keystone species, we could imagine that small inclusion of some species could have a relatively bigger impact on interactions than suggested by their proportion in the mixture (Bond, 1994). Connolly *et al.* (2013) showed that interaction between species could reach a plateau in its central part, and the proportion necessary to reach this plateau could have strong implications in the design of mixtures. Such shape in species interactions could be estimated through the inclusion of the power of the proportions of the species involved in the interaction (θ) (Connolly *et al.*, 2013). However, using the data from Chapter 2, extra complications (asymmetric, ternary or powered interactions) did not improve the models (based on AICc) for predicting annual yield. Due to the level of detail implicated for such precision in the BEF relationship, an experiment should be designed specifically for this question. A high number of mixtures with emphasis on unbalanced proportions could help to disentangle if the inclusion of any extra ternary, asymmetry parameter or power coefficients would be relevant in the assessment of interactions.

Future work

Design adjustments for deeper characterization of interactions

To tackle the limitations about pairwise interactions assessment, and investigate further optimization of diversity effect on ecosystem functioning, some modifications could help the design. Communities of two and three species from two and three different functional groups respectively would bring a better knowledge of the interactions between species from different FG, when in this work they were grouped in global ‘between FG interactions’ (Fig. 5.2). These extra treatments (n=20 in a six-species pool design) would not necessarily need to be replicated as they add to the spread of points across the shape and help reducing extrapolation distance. Thus, they would be a reasonable addition to a similar design; that could help answering an interesting research questions: is there redundancy in interactions across species or FG? Its straight application in agriculture can be formulated through the consequent question: can the BEF relationship be optimized at low species richness? This is one of the purposes of the LegacyNet experiment designed by Brophy *et al.* (2021). It consists in adding the communities discussed above to the design of the present experiment (without including the drought treatment).

The conditions for lower species communities to optimize performance requires transgressive overyielding at low diversity. As discussed above, the conditions are that pairwise interactions are strong, specifically between the highest performing species. In case one species produces much larger outcome, the occurrence of transgressive overyielding would be less likely. One monoculture outperforming other community can occur in sites where conditions (environment and management) are particularly favourable for one species. To overcome this potential limitation, LegacyNet is a network of 34 experimental sites around the world. The multi-site analysis will help generalize conclusions about the BEF relationship.

Developing visualization tools for linking the complex definition of diversity with multiple dimensions of agro-ecosystems outcomes can be challenging. To overcome the limits associated with representations in two dimensions, an interactive tool is under development to visualize grassland multifunctionality, adjust weighing across functions and optimize species composition and proportions for a given weighing (built with data from Chapter 4).

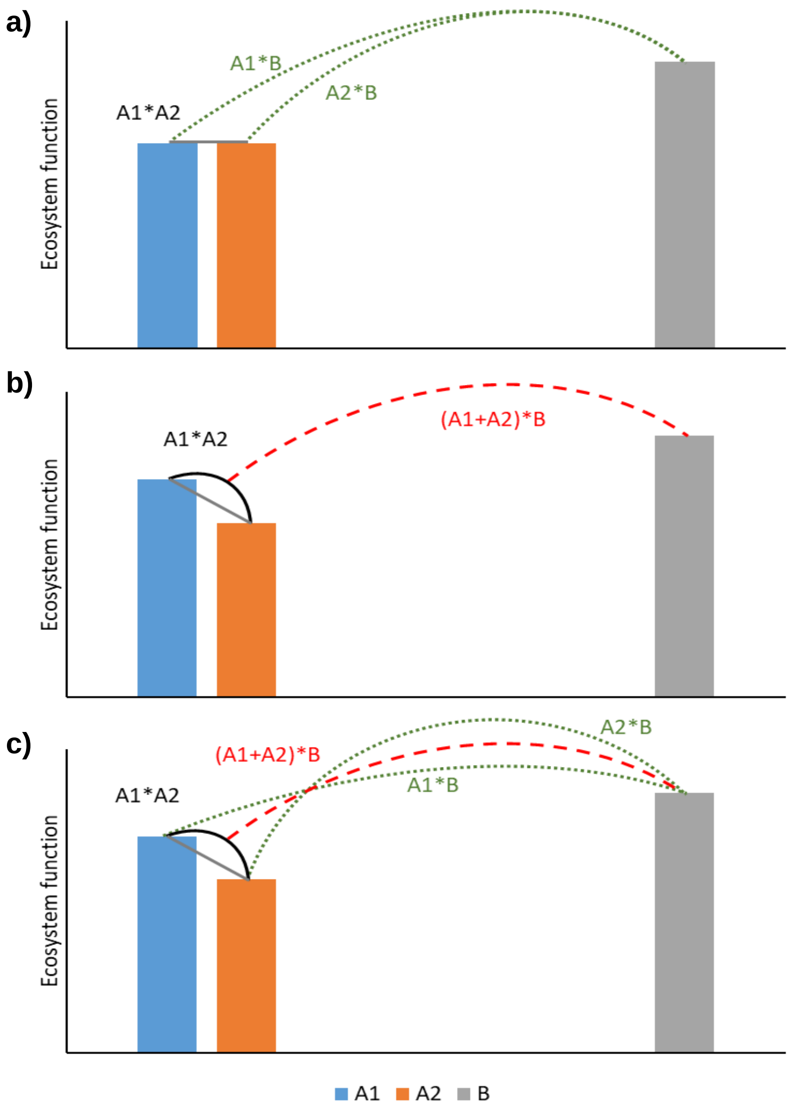

Figure 5.2. Conceptual graphs illustrating the importance of knowledge about species interactions. This hypothetical example includes a functional group (A) containing two species (A1 and A2: blue and orange bars respectively) and a second functional group (B: the grey bar). The A1 by A2 interaction (within-FG interaction, A1*A2) is represented by a continuous line: grey and flat if A1*A2=0, black if A1*A2>0 with the height being driven by the strength of the interaction. The dashed colour lines show the interactions between the two functional groups: green at the species level (A1*B or A2*B) and red at the functional group level (A*B). The heights of these lines are driven by the strengths of the various interactions. Panel (a) shows the required conditions for FG redundancy between species A1 and A2 which are: identical performance from the two species (A1=A2), no interaction between these species (A1*A2=0), and that species interact the same with others (A1*B=A2*B). Panel (b) shows the interactions characterized in this thesis. Keeping the same identity and between-FG interaction effects, panel (c) shows a case where despite species A2 performing lower as monoculture, the highest performance is achieved by a mixture of A2 and B. This panel points the potential importance of characterizing interactions at pairwise interaction level.

Prepare better for climate change

The maximization of the BEF relationship is a challenging exercise, as it can shift across functions or in case of changes in abiotic factors (Grant *et al.*, 2014). I showed that drought affected ecosystem functions differently, with some functions being reduced uniformly across all communities. This was notably the case for the legacy effect which indicates the importance of considering the residual effect of drought over a longer term (e.g. 6-24 months) than the growing season in which it occurred (Chapter 3). Such observation suggests important lessons for methodology to assess the resilience of agro-ecosystems. Indeed, if a residual effect of drought is to be expected, what about the effect of repeated summer droughts or alternations with winter waterlogging? Another aspect of the longer term assessment of a disturbance is whether the system has a 'memory' of the disturbance, i.e. does a previously stressed community cope better with drought? Several studies investigated these aspects and found plant metabolic adaptations to induce 'memory' of stress in pot experiments under controlled environments (Tombesi *et al.*, 2018; Wojtyla *et al.*, 2020). For upscaling conclusions to the agro-ecosystem, a plot-scale experiment would be required, but the multiplication of modalities would result in a logistically challenging setup (high number of plots, duration, equipment for stress simulation, etc.). However, a double split-plot design could be built, learning from the design of the work presented her. After a first year of drought simulation (with plots randomly split in two halves on the North-South direction), the rainout shelters could be applied perpendicularly the second year (on the East-West direction). This would result in four different water supply treatments (Fig. 5.3).

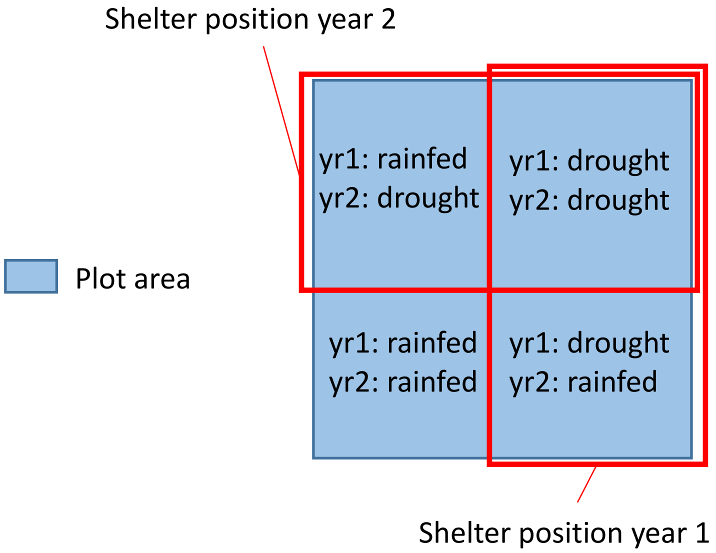

Figure 5.3. Plot layout suggested for the assessment of the residual effect of summer drought on perennial grassland. Red rectangles represent shelter positions for the two consecutive summers. The plot would then be split in four parts, each part counting a discarded edge and a central harvested part for measurements.

The results from an experiment manipulating diversity and crossing it with fluctuations of drought stress across two years would enable to answer several research questions under 'real-farm' management:

- Is there a 'memory' of drought, i.e. is there a significant difference between the two sub-plots where drought is applied in year 2, thus associated to previous year water supply?
- Is there a residual effect of drought on the subsequent year in a perennial grassland (as I show in Chapter 3, but without a change in crop)?
- Can we draw a parallel between water supply and shifts in species proportions?
- Such an experiment could also be the occasion to investigate belowground processes (effect of drought on root growth and exudates, decomposition rates, microbial activity, nutrient cycles, etc.).

The crossed plot layout (Fig. 5.3) could also be adapted to cross drought with another abiotic factor. To do so, the second year treatments could be replaced by a gradient of this abiotic factor. Thus, two levels (or more, if plot size is sufficient) of winter waterlogging, fertiliser rates or types, cutting height or frequency, mechanical compaction, etc. could be tested. Such an experiment would be a great opportunity to study the effect of combined disturbances, which is an area with clear lack of information, as pointed out by Hong *et al.* (2022).

In Chapter 3, I investigated the potential effect of mineral fertiliser on the follow-on crop. In reviews by Smith and Chalk (2018) and Edmeades (2003), the studies investigating the residual effect of fertiliser in perennial grasslands are not common, they rarely included a control, and none crossed fertiliser effect with plant diversity. Substituting the assessment of drought by two levels of fertiliser across two years (Fig. 5.3) would first allow to quantify the effect of increased fertiliser across all monocultures and mixture. Second, it would enable tracking mineral fertiliser residual effect on the follow-on growing season, across communities of varying diversity. Third, this would generalize the comparison between the effects of increased diversity and increased inputs, instead of constraining it to the same starting point (Fig. 3.5).

One limitation of the assessment of drought effect on grassland is the reduction in photosynthetic Active Radiation (PAR) embedded with the use of rainout shelters, as discussed in Chapters 2, 3 and 4. One way to isolate the effect of drought only from other factors could be to cover the whole experimental field, irrigating only the 'rainfed' plots. Although such design is common for pot scale experiments (e.g. Xu *et al.*, 2022), it would be challenging to implement at plot scale, given the area concerned. In order to minimize the effect of the shelter on other aspects than the sole soil moisture, experimental setups were developed using automated mobile shelters that would stand aside the experimental plots, covering the drought treatment only during rainfall events (Beauchêne *et al.*, 2019). Given the increasing concerns about climate variability, such design could be relevant to implement, despite the costs associated.

Implement experimental results on real farm

Several group of farmers visited the experiment and were curious about application of results to real farm. Recurrent questions rose about the persistence of mixtures under grazing conditions and the milk and meat production potential while maintaining animal health. Promising results were published about the effect of plant diversity on several aspects of pastures such as yield, stability, response to water supply, utilization or weed invasion (Skinner *et al.*, 2004; Tracy and Sanderson, 2004; Sanderson, 2010a; Sanderson, 2010b). Other works showed the potential of multi-species swards to promote grazing animals' growth, dairy production or low parasitism (Soder *et al.*, 2006; Soder *et al.*, 2007; Cranston *et al.*, 2015; Roca-Fernandez *et al.*, 2016; Bryant *et al.*, 2017; Grace *et al.*, 2018). However, among the multitude of experiments, few assessed the persistence of multi-species swards over a pasture lifespan. In addition, pasture management can have a strong effect on grassland properties, thus local and recent references are required (Sanderson *et al.*, 2004). Finally, as reviewed by McCarthy *et al.* (2020), the majority of studies about the effect of inclusion of new species in dairy animal diet do not consider the full lactation, nor the addition of concentrate feed.

To accurately assess the potential of multi-species mixtures on real farms, a grazed trial is ongoing on both dairy and beef units of Teagasc, Johnstown Castle experimental farm, since 2020 (Fig. S5.1). To compare high-diversity, low-input management to low-diversity, high-input, two homogeneous 7.5 ha and 20 dairy cows farmlets are managed as closed systems. One farmlet was sown with a six-species mixture of 60% grass, 25% legume and 15% herb, receiving an annual fertiliser input of 80 kg N ha^{-1} yr^{-1}; the other farmlet was sown with a 90% *L. perenne*, 10% *T. repens* mixture, receiving 200 kg N ha^{-1} yr^{-1}. Dairy cows are fed from grazed pasture or silage exclusively from the farmlets, adjusting with concentrate feed to meet animals' requirements. Management is kept as similar as possible between the two farmlets. All inputs and outputs are measured, as well as regular assessment of sward production and composition. By keeping these systems in parallel for at least five years, it is intended to disentangle any dissimilarity about systems input or output. Species proportions in the multi-species paddocks will be assessed after few years of trial, to answer queries about persistency. Any required adjustment in management will be noted, as it is of great interest for farmers. This way, local and up to date references will be produced about on-farm implementation of multi-species swards. This concrete application of the experimentally tested BEF relationship has potential for increasing farm biodiversity and resilience, maintaining system outputs and reducing reliance on external inputs. Such on-farm references are crucial in a context of national scale growing interest for implementation of multi-species swards (Gilsenan, 2019; Germinal, 2020; DLFseeds, 2021; Kelleher, 2021; McDonnell, 2021; Walsh, 2021).

Conclusion

This study demonstrates that multi-species swards have a great potential to tackle challenges associated with intensively managed grassland monocultures. Indeed, I showed that plant diversity can mitigate the consequences of an extreme weather event, while stimulating ecosystem functioning. The higher agronomic performance of diverse swards fulfil the gaps identified in O'Donovan et al. (2022), who recognized plant diversity and lower reliance on fertiliser inputs as priorities for future management of intensive grasslands in Ireland. The proven potential of multi-species swards at plot scale, now needs to be turned into practical management recommendations for livestock systems, to promote economic and environmental results.

Supplementary material

Figure S5.1. Dairy herd grazing a multi-species sward in Johnstown Castle experimental farm (September 2020).

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