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

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Abstract

1. 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.

2. 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 2-month experimental drought, were applied. Mineral nitrogen fertiliser was applied at 150 kg ha⁻¹ year⁻¹, and a *Lolium perenne* monoculture received 300 kg ha⁻¹ year⁻¹ (300N) as a highly fertilised comparison. Annual above-ground biomass was measured for 2 years.

3. 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.

4. 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.

5. 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...
1 | 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 (Falzo 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 (Lüscher et al., 2014; Trenbath, 1974). This is achieved through multiple synergistic interactions among species, including complementarity in resource use (e.g. acquisition of soil nutrients and water; symbiotic N₂ fixation and utilisation of light). Multi-species mixtures in intensively managed systems have been shown to achieve not just overyielding (Moloney et al., 2020; Sanderson et al., 2013) 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 3-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 (one-, two- and four-species mixtures from a species pool of one grass, two legumes and one herb) to higher yield, greater drought resistance and higher yield stability. Komandza 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 year⁻¹ of N) on L. perenne (300N L. perenne) was included for comparison with all other communities that received 150 kg ha⁻¹ year⁻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 Figure 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 a well-established statistical theory for this type of data (see Cochran & Cox, 1957).

The specific aims of this 2-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 the impact of drought 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 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 $y = \beta_1 P_1 + \beta_2 P_2 + \delta P_1 P_2 + \epsilon$ (adapted from Equation 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 $\beta_1$ and this is the expected performance of species 1 in monoculture (similarly it is $\beta_2$ 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 ($\delta P_1 P_2$). The expected ecosystem function for the 70:30% mixture is $\beta_1 \times 0.7 + \beta_2 \times 0.3 + \delta \times 0.7 \times 0.3$. In this example, species 1 performs better than species 2, and so has a higher identity effect; and $\delta$ 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, that is, where a mixture outperforms the weighted monoculture performance. The horizontal dashed line shows the ‘transgressive overyielding’ threshold, that is, where a mixture outperforms the best-performing monoculture.

2 | MATERIALS AND METHODS

2.1 | 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 of soil, respectively) was ploughed and reseeded with a total of 43 plots, each of area 35 $m^2$ (5 m × 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 S1.

Communities received 150 kg/ha of nitrogen fertiliser per annum (150N), split into five applications. Four replicates of a *Lolium perenne* monoculture receiving 300 kg/ha of nitrogen were included as an extra community for a high fertiliser comparison (300N). Background fertilisation of 60 kg/ha of phosphorus and 300 kg/ha 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 × 3.5 m split plots. One half (randomly chosen) received natural water supply over the year (‘rainfed’), while a 2-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).

2.2 | 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 10 years (2013 and 2018). Moreover, climate models predict a 12%–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 Figure S1). Air temperature and humidity were recorded using Tynitag© TGP-4500.
monitors. No significant difference was measured in air temperature or humidity in and out of the shelters. To monitor soil water availability, soil water potential probes were installed at 10 and 40 cm below-ground 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 (Figure 2). Due to exceptionally low rainfall 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 is found in Table S2.

2.3 | 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 Figure 2). From a central 1.5 m strip of each plot, fresh biomass was weighed by the harvester, and dry matter content was determined by oven drying a representative sub-sample of approximately 100 g. Dry matter yield was summed for each year to obtain total annual above-ground biomass production and 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 2 years. The following linear mixed model was fitted to the observed experimental data:

\[
\text{DMY} = \text{Year} \times \text{Treatment} \times \left[ \beta_1 P_{\text{Lp}} + \beta_2 P_{\text{Pp}} + \beta_3 P_{\text{Tp}} + \beta_4 P_{\text{Tr}} + \beta_5 P_{\text{Ci}} + \beta_6 P_{\text{Pi}} + \beta_7 P_{\text{Ci}} P_{\text{Pi}} P_{\text{Tr}} + \delta_{\text{GL}} P_{\text{Ci}} P_{\text{Pi}} P_{\text{Tr}} + \delta_{\text{LS}} (P_{\text{Lp}} + P_{\text{Pp}}) (P_{\text{Ci}} + P_{\text{Pi}}) + \delta_{\text{CS}} (P_{\text{Lp}} + P_{\text{Pp}}) (P_{\text{Ci}} + P_{\text{Pi}}) + \delta_{\text{GH}} P_{\text{Lp}} P_{\text{Pp}} + \delta_{\text{PS}} P_{\text{Lp}} P_{\text{Pp}} + \delta_{\text{PC}} P_{\text{Lp}} P_{\text{Pp}} + \delta_{\text{PS}} + \delta_{\text{PC}} + \gamma St + \epsilon \right],
\]

where DMY was the annual dry matter yield, \( P \) was the sown proportion of a species (with subscripts \( \text{Lp} = L. \ perenne, \text{Pp} = P. \ pratense, \text{Tp} = T. \ pratense, \text{Tr} = T. \ repens, \text{Ci} = C. \ intybus, \text{Pi} = P. \ lanceolata \) for

In a hypothetical three-species situation, a Diversity-Interactions model may take the form:

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

If \( P_1 = 1 \), \( P_2 = 0 \) 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 your data, even if the exact set of values are not included in the dataset. Thus, our method has the advantage (over ANOVA-based approaches for BEF studies) of allowing estimation of the performance of communities that were not included in the field experiment but are encompassed by the design space.

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 (Equation 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 2 years. The following linear mixed model was fitted to the observed experimental data:

**Figure 2** Volumetric soil moisture content (%) at 10 cm (a) and 40 cm (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 ~1.5 MPa. It is considered that plant water absorption is obliterated below this threshold (permanent wilting point). Vertical lines represent harvest dates.
plots fertilised under the 150N regime. Each $\beta_i$ term stands for a species’ identity effect (explained for a two-species system in Figure 1), that is, the expected annual dry matter yield for a monoculture of species $i$ under 150N fertiliser ($P_i = 1$, $P_j = 0$) is $\beta_i$, and when $P_i < 1$, the species identity contribution to the mixture is $P_i \beta_i$. The extra factor $X_{ij}$ was coded 0 and 1 for sub-plots that received 150N and 300N fertiliser, respectively. There were only eight sub-plots (four rainfed and four drought) that received the 300N fertiliser, and each were monocultures of L. perenne. The covariate $P_{1p}$ was coded 0 for these high N sub-plots, therefore $γ$ is the expected average yield of 300N L. perenne monocultures. We restrict our inference related to this high N treatment, that is, 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 ($G = \text{grass}, L = \text{legume}, H = \text{herb}$) 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 ($δ_{GC}$ or $δ_{HL}$) or restricted maximum likelihood (for comparisons of error structures and final model presentation).

The final Diversity-Interactions model selected was:

$$
\text{DMY} = \text{Year} \times \text{Treatment} \times \{ \beta_1 P_{1p} + \beta_2 P_{2p} + \beta_3 P_{3p} + \beta_4 P_{4p} + \beta_5 P_{5p} + \beta_6 P_{6p} \} \\
+ \text{Year} \times \{ \delta_{CC} P_{1p} P_{2p} + \delta_{CH} P_{1p} P_{3p} + \delta_{NH} P_{4p} P_{5p} + \delta_{CL} P_{1p} P_{6p} + \delta_{CH} P_{2p} P_{6p} + \delta_{NH} P_{3p} P_{5p} \} \\
+ \delta_{GN} (P_{1p} + P_{2p}) (P_{3p} + P_{4p}) + \delta_{HN} (P_{5p} + P_{6p}) (P_{3p} + P_{5p}) \} \\
+ \text{Year} \times \text{Treatment} \times \{ \gamma X_{ij} \} + \varepsilon.
$$

Equation 2 varies from Equation 1 only in that the interaction terms ($δ_{CC}$, $δ_{CH}$, $δ_{NH}$, $δ_{CL}$, $δ_{CH}$, and $δ_{HN}$) were not crossed with the water supply treatment. The AICc was reduced by 40.5 for the fitted model in Equation 2 compared to Equation 1. Alternative assumptions about the interactions did not improve the model AICc; this included testing for nonlinearity 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 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 1, we get $\text{DMY} = 11.7 \times 0.5 + 10.3 \times 0.5 + 2.9 \times 0.5 + 0.5 = 11.7$. All other species proportions and $X_{ij}$ 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 Section 2).

### 3.2 Comparison of species’ yields

The species identity estimates (Table 1a) can be interpreted as the monoculture performance for each species; for example, the annual yield of a L. perenne monoculture under rainfed conditions in 2018 was 9.2 t/ha. The species’ identity effects also feed into prediction for mixture communities as a weighted average of the component species (Figure 1); for example, the identity contribution of L. perenne to a mixture under rainfed conditions in year 1 is $9.1 \times P_{1p}$ t/ha, where $P_{1p}$ is the sown proportion of L. perenne in the mixture.

Under rainfed conditions, the highest-yielding monoculture was T. pratense (11.7 t/ha) in 2018 and P. lanceolata (10.5 t/ha) in 2019 (estimated by the species identity terms in Table 1a, and see Figure 3). Under drought, the highest-yielding monoculture was 300N L. perenne in both years (10.4 t/ha in 2018 and 2019), followed by P. pratense (10.1 t/ha) in 2018 and P. lanceolata (10.2 t/ha) 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 instead of 1.4 t/ha in 2018, in average across 150N monocultures. Different species were affected, with L. perenne, P. lanceolata and 300N L. perenne not showing a significant difference between rainfed and drought.

### 3.3 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 and legume-herb) were strong and positive in each year (Table 1a, see FG interaction estimates); those involving legumes were particularly strong. None of the interactions between species from the same FG were significant.
| 2018 | 2019 | 2-year average |
|------|------|----------------|
|      | Rainfed | Drought | Diff | Rainfed | Drought | Diff | Rainfed | Drought | Diff |
| 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
|      |       |       |      |       |       |      |       |       |      |
| FG interaction (β) | | | | | | | | | |
| Grass–Grass | 0.5 | ±0.13 | | | | 1.4 | ±0.14 | | | |
| Legume–Legume | 2.9 | ±0.10 | | | | 2.9 | ±0.13 | | | |
| Herb–Herb | −3.0 | ±0.10 | | | | −1.0 | ±0.13 | | | |
| Grass–Legume | 6.1 | ±0.75 | | | | 6.4 | ±0.18 | | | |
| Herb–Herb | 4.8 | ±0.75 | | | | 3.7 | ±0.18 | | | |
| Legume–Herb | 5.8 | ±0.72 | | | | 7.3 | ±1.17 | | | |
| 300N L. perenne (γ) | 10.7 | ±0.41 | 10.4 | ±0.41 | ns | 10.3 | ±0.32 | 10.4 | ±0.32 | ns |
| Monoculture average | 10.3 | ±0.17 | 8.8 | ±0.17 | *** | 9.4 | ±0.14 | 8.6 | ±0.14 | *** |
| Six-species centroid | 12.1 | ±0.23 | 10.7 | ±0.23 | *** | 11.6 | ±0.19 | 10.8 | ±0.19 | *** |
| Four-species Grass–Legume | 12.3 | ±0.42 | 10.6 | ±0.42 | *** | 11.3 | ±0.35 | 10.4 | ±0.35 | *** |
| Four-species Grass–Herb | 10.9 | ±0.42 | 9.9 | ±0.42 | *** | 10.4 | ±0.35 | 9.7 | ±0.35 | *** |
| Four-species Legume–Herb | 11.7 | ±0.42 | 10.1 | ±0.42 | *** | 11.7 | ±0.35 | 10.9 | ±0.35 | *** |
| Five-species (excluding L. perenne) | 12.3 | ±0.26 | 10.4 | ±0.26 | *** | 11.8 | ±0.22 | 10.9 | ±0.22 | *** |
| Five-species (excluding P. pratense) | 11.9 | ±0.25 | 10.4 | ±0.25 | *** | 11.5 | ±0.21 | 10.8 | ±0.21 | *** |
| Five-species (excluding T. pratense) | 11.6 | ±0.26 | 10.4 | ±0.26 | *** | 11.4 | ±0.22 | 10.7 | ±0.22 | *** |
| Five-species (excluding T. repens) | 11.9 | ±0.26 | 10.5 | ±0.26 | *** | 11.1 | ±0.22 | 10.3 | ±0.22 | *** |
| Five-species (excluding C. Intybus) | 12.6 | ±0.26 | 10.8 | ±0.26 | *** | 11.8 | ±0.22 | 11.0 | ±0.22 | *** |
| Five-species (excluding P. lanceolata) | 12.1 | ±0.26 | 10.8 | ±0.26 | *** | 11.4 | ±0.26 | 10.4 | ±0.26 | *** |

**TABLE 1** Parameter estimates (a) and predictions from selected communities (b) from the mixed model shown in Equation 2; the units are t ha⁻¹ year⁻¹. The standard errors for the 2-year average values take year-to-year covariance into account. The Diff columns give the level of significance of the difference between the rainfed and drought treatments (***p ≤ 0.01, **p ≤ 0.05, *p ≤ 0.1, ns: not significant). In part (a), grey highlights fixed effects not crossed with drought. 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 2-year average (only), hash signs (#) denote the transgressive overyielding, that is, communities significantly outperforming P. pratense, the highest-yielding monoculture (α = 0.05). Italic is used for species name in latin and for easier reading of standard errors (to avoid mixing with estimates).
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 (Figure 4). Mixtures including two functional groups or more, with balanced species’ proportions, had a consistent positive interaction effect, ranging from $+0.8$ t/ha to $+2.4$ t/ha, depending on year and community. As noted earlier, including legumes with other FGs was particularly beneficial, whether mixed with grass, herb or both (Table 1a, FG interaction terms and Figure 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 (Equation 2). This means that although mixture yields were generally reduced by drought (Table 1b),...
FIGURE 5 Contour plots showing the model predictions of annual dry matter yield (t ha\(^{-1}\) year\(^{-1}\)) from any mixture sown with grass, legume and/or herb under rainfed conditions (a, c, e, g) or drought conditions (b, d, f, h). Annual yield was predicted across the three-dimensional functional group simplex space for rainfed and drought for 2018 (a and b), 2019 (c and d) and the average of the 2 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 2-year average, taking year-to-year covariance into account. The white area represents where 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 standard errors (SE). Thus, the area in black displays ‘significant’ results of a conservative test for transgressive overyielding.
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 due to interactions under drought compared to the corresponding monocultures.

3.4 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 2 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, 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 (Figure 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 as follows: grass–legume = 11.8 t/ha; grass–herb = 10.7 t/ha and legume–herb = 11.7 t/ha (Table 1b). Thus, on average over the 2 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, Figure 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 (Figure 5 and see Kirwan et al., 2009).

Using the estimates from the model (Equation 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 (Figure 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 (Figure 5a–f).

3.5 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 (Figure 5g,h, on average over the 2 years). Averaged over the 2 years, transgressive overyielding was attained by all four- and five-species equi-proportional mixtures, except the four-species grass–herb mixture (Table 1b, selected communities denoted by #).

3.6 Yield comparisons with the 300N L. perenne monoculture

Averaged over the 2 years and under rainfed conditions, the 300N L. perenne monoculture overyielded the 150N L. perenne monoculture by 1.3 t/ha (10.5–9.2 t/ha; p < 0.001, Table 1). Simultaneously, the six-species equi-proportional mixture (centroid) overyielded the 300N L. perenne by 1.3 t/ha (11.8–10.5 t/ha; 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 2 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, 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.

4 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.

4.1 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 (Figure 4) resulted in transgressive overyielding (outperforming the highest-yielding monoculture) for a wide range of plant communities in the design space (Figure 5g,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 Cong et al., 2018; Grace et al., 2018; Moloney et al., 2020; Sanderson, 2010). 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 1a). Thus, legume–herb mixtures yielded similarly to grass–legume mixtures, on average over the 2 years (Table 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 (Hoeckstra et al., 2014) and asynchrony of growth (Haughey et al., 2018; Husse et al., 2016), 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 & 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 optimise the multiple benefits of herb inclusion in intensively managed grasslands.

### 4.2 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 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).

### 4.3 Practical implications

#### 4.3.1 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 contributes 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 diversity mitigated the impact of an extreme weather event in intensively managed grasslands. This is important, in the context of a predicted 12%–40% increase in summer dry periods by 2050 (Nolan et al., 2017).

#### 4.3.2 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 N₂O emission intensity (g of N₂O per kg of dry matter yield) when comparing the six-species centroid with 300N L. perenne (Cummins et al., accepted).

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 (Cummins et al., accepted; Dooley et al., 2015; Grange et al., unpublished; Suter et al., 2021). 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.

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AUTHORS’ CONTRIBUTIONS
C.B. and J.A.F. conceived the ideas and designed the methodology; G.G. collected the data; G.G., C.B. and J.A.F. analysed the data; G.G., C.B. and J.A.F. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT
Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.qfttdz0g9 (Grange et al., 2021).

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