European Journal of Agronomy 138 (2022) 126531



Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja





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

Guylain Grange [a,b,*], Caroline Brophy [a], John A. Finn [b]

[a] School of Computer Science and Statistics, Trinity College Dublin, Dublin 2, Ireland
[b] Environment, Soils and Land Use Dept., Teagasc, Johnstown Castle, Wexford Y35 TC97, Ireland

ARTICLE INFO

Keywords:
Crop
Diversity
Grassland
Nitrogen
Rotation
Yield
Multispecies
Fertiliser
Mixture

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 sown 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.

1. 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 (Van der 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

* Corresponding author at: Environment, Soils and Land Use Dept., Teagasc, Johnstown Castle, Wexford Y35 C97, Ireland.
E-mail address: grangeg@tcd.ie (G. Grange).

https://doi.org/10.1016/j.eja.2022.126531
Received 2 November 2021; Received in revised form 28 March 2022; Accepted 26 April 2022
Available online 26 May 2022
1161-0301/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

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, the 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. 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.

2. Materials and methods

2.1. 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.

2.2. 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 A.1). These plots received an annual fertiliser rate of 150 kg ha^{-1} of nitrogen (150 N), 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 (300 N) applied, as a high N comparison. The location of the 43 main plots was randomised and each 7 m x 5 m main plot was split into two 3.5 m x 5 m 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 plots 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. During each of 2018 and 2019, all plots were harvested on seven occasions, cutting the central strip of each sub-plot (1.5 m x 5 m) at 4.5 cm 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.

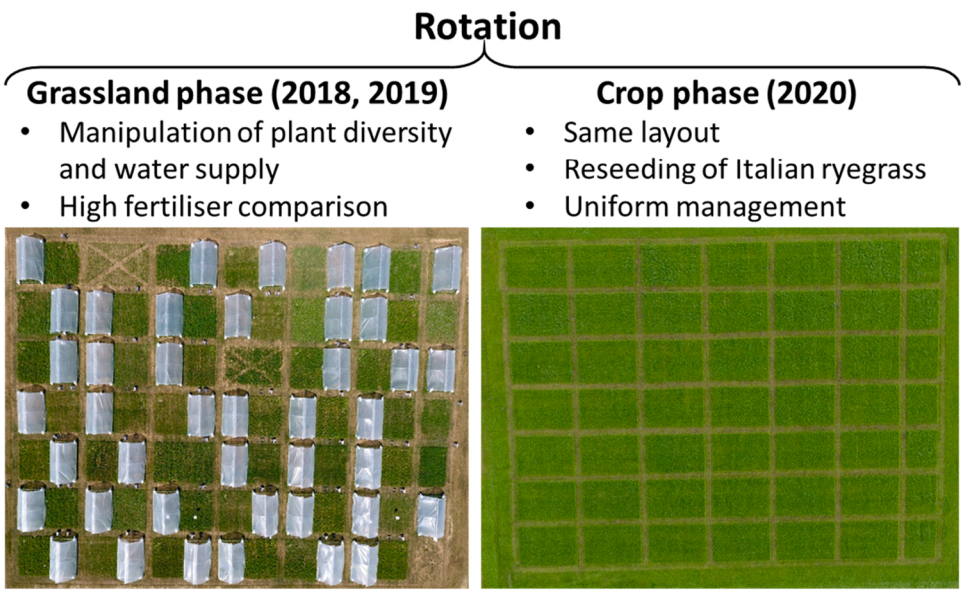


Fig. 1. Drone image overview of the experimental design and plot management to track the effect of plant diversity, drought and fertiliser level on the legacy effect within plots. First, we manipulated grassland diversity and water supply during a two-year grassland phase and included a 'high nitrogen 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.

2.3. 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. A.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. The DMY and NY from the follow-on crop is used to estimate the relative effects of plant diversity, drought, and 300 N on legacy effects.

2.4. 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 Eq. 1:

$$y = \left(\sum_{i=1}^{6} \beta_i P_i + \sum_{\substack{i,j=1 \\ i<j}}^{6} \delta_{ij} P_i P_j + \gamma X_N \right) * Drought + \varepsilon \quad (1)$$

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, represents 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 150 N ($X_N = 0$) and 300 N ($X_N = 1$) 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 A.2.

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. A.2).

3. 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 A.2, the best model for both responses (total dry matter yield (DMY) and nitrogen yield (NY)) was:

$$y = \sum_{i=1}^{6} \beta_i P_i + \gamma X_N + \eta X_D + \varepsilon \quad (2)$$

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 δ_{ij} different from 0, Eq. 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 (Eq. 2). The 300 N 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 300 N *L. perenne* monoculture, and

$\gamma + \eta$ is the expected y for drought 300 N *L. perenne* monoculture. The legacy effect of any community, including mixtures at 150 N, 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 Eq. 2 can be expressed to two ways, plugging in $X_N = 0$ for any monoculture or mixture at 150 N gives: $\sum_{i=1}^{6} \beta_i P_i + \eta X_D$, and plugging in $X_N = 1$ for 300 N *L. perenne* monoculture gives: $\gamma + \eta X_D$ (since all $P_i = 0$ in this case). (It would be inappropriate to extrapolate the 300 N effects for communities other than *L. perenne* monoculture.).

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

Among the six monocultures of the tested grassland species, the two legume species resulted in the highest dry matter and nitrogen DMY and NY of Italian ryegrass in the crop phase (Table 1 and Fig. 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 (Eq. 2) meant that mixtures were predicted as the weighted average of component species. The examples given in Table 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. 3b.

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

During the grassland phase, 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. 2, Table 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. A.3).

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 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. 3b).

Compared to all other communities, lowest values of DMY and NY in the crop phase were from the 300 N *L. perenne* monoculture (although not significantly different from 150 N *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 300 N *L. perenne* monoculture, including mixtures without a legume contribution (e.g. Table 1b). For example, the equi-proportional mixture of the two species with lowest legacy effect (*L. perenne* and *P. lanceolata* at 150 N) would lead to a higher legacy effect than that from 300 N *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.

3.3. 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. 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.

Table 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 (Eq. 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 by 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*
300 N *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*

4. 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).

4.1. 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

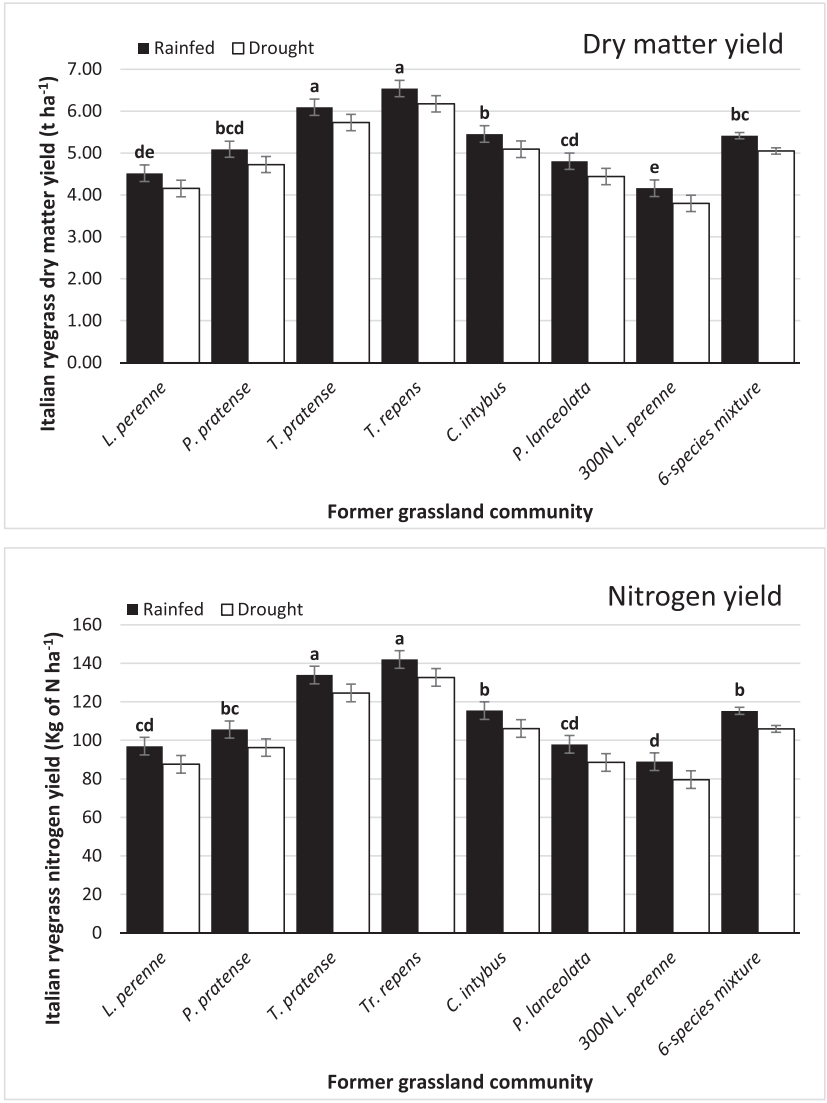


Fig. 2. Modelled predictions from the Eq. (2) estimates (Table 1a) for dry matter (a) and nitrogen yield (b) of an Italian ryegrass (*L. multiflorum*) monoculture depending on the previous grassland plant community. Shown are the predicted monocultures for each species at 150 N, the predicted 300 N *L. perenne* monoculture, and the equi-proportional 6-species mixture at 150 N. Note that any mixture community at 150 N can similarly be predicted from the model. The 300 N *L. perenne* monoculture acts as a high N comparison to the regular 150 N *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 ($\alpha = 0.05$). Communities that are different for rainfed (black bars) are also different for drought (white bars), as the drought effect was constant across all communities.

water supply (weather data displayed in Fig. A.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 properties. 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 mineralisation 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.

4.2. *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 over-yielding in legacy effects (i.e. greater yield in crop preceded by mixture than weighted combination of the legacy effect of the component species in monoculture). 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). In Fig. 5, we show that the six-species grassland

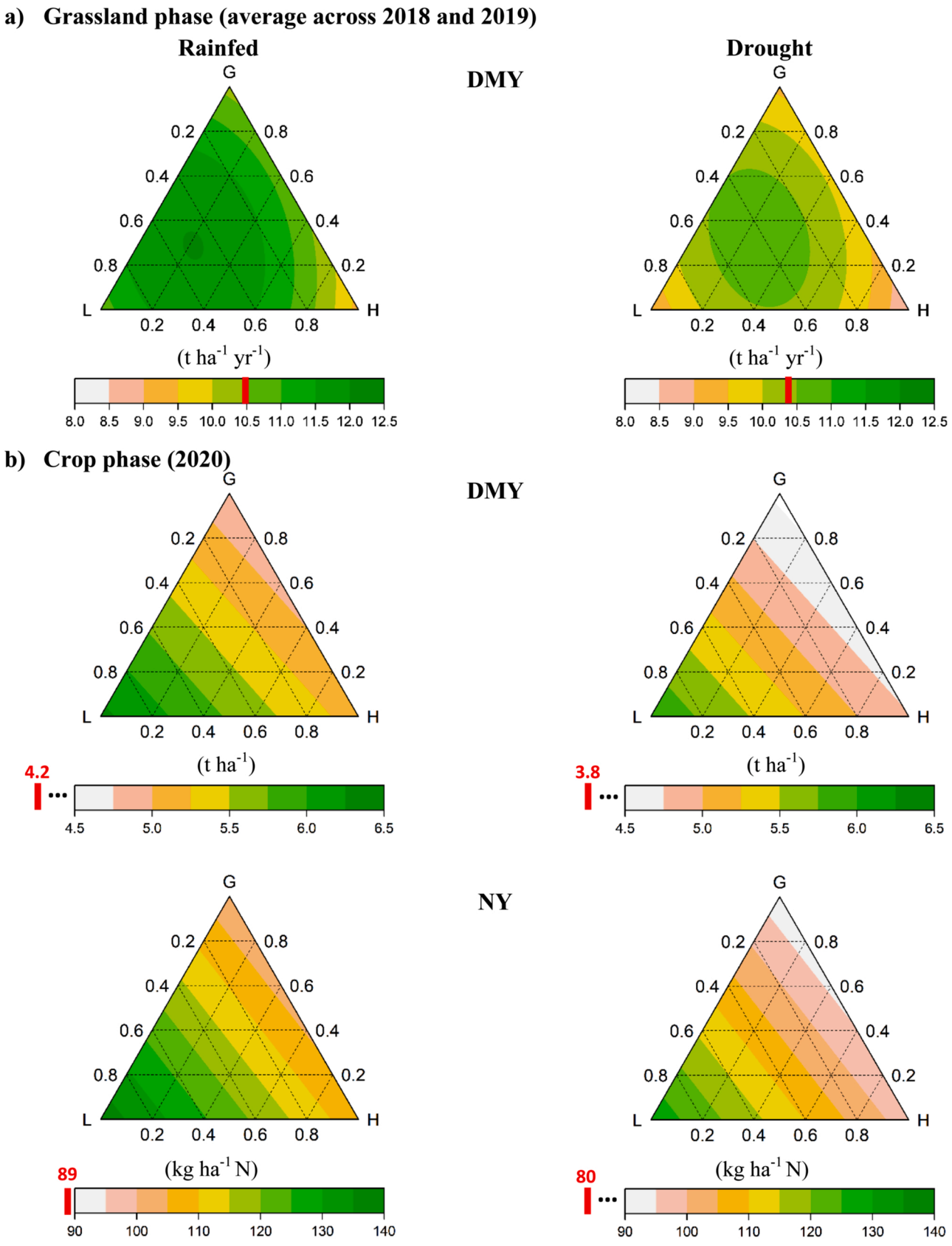


Fig. 3. Effect of functional group composition (relative proportion of grasses, legumes and herbs) on average annual dry matter yield (DMY) over two years in the grassland phase (panels a; from Grange et al. 2021), and DMY and total NY (b) of *L. multiflorum* in the one-year crop phase. Predictions for 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 300 N *L. perenne* monoculture; where it is out of the legend range, the value is included in text above the red bar.

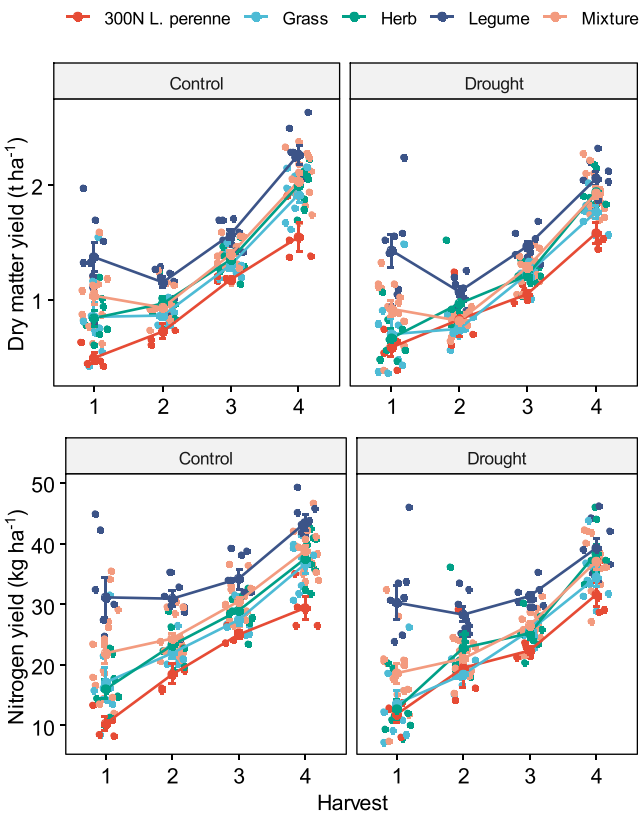


Fig. 4. Harvest-scale raw data for DMY and NY of an Italian ryegrass crop depending on community composition of the preceding grassland (300 N *L. perenne*, 150 N: grass only, herb only, legume only or mixture of at least two functional groups). Bars represent standard errors. The average point for each community type is connected for each harvest to show the trends in each response over time (four harvests from mid June to late September (see Fig. A.1)).

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 1b, Fig. 3b).

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.

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

Increased nitrogen fertiliser input in *L. perenne* monoculture grassland (300 N) did not result in a higher performance of the follow-on crop in comparison to a lower fertiliser rate (150 N). In addition, the extra nitrogen applied to the 300 N 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 (Figs. 3b, 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. 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.

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

We have focused on the results from the follow-on crop phase. 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. 3a, from Grange et al. (2021)), but overyielding due to legacy effects did not occur during the crop phase (Fig. 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

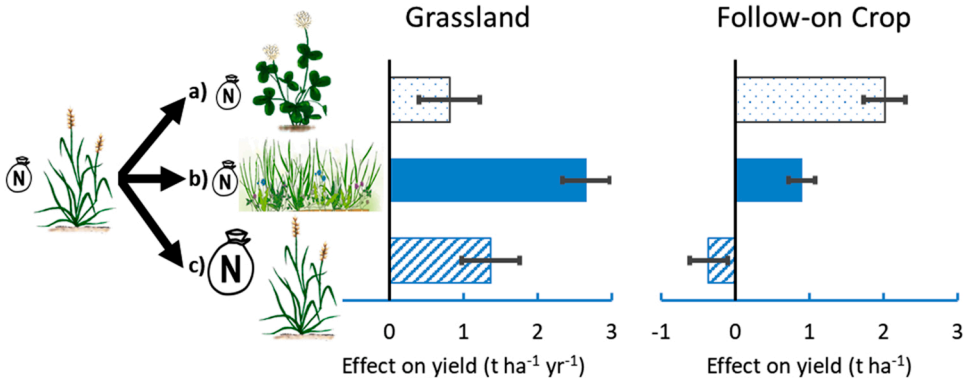


Fig. 5. Comparison of alternative changes in grassland management practice on mean (± SE) dry matter yield in both the grassland and follow-on crop. Relative to 150 N *L. perenne* monoculture (low-diversity, low-input), we compare the effect on yield across a grassland-crop rotation of (a) a 150 N *T. repens* clover monoculture (the community with the highest legacy effect); (b) the six-species equi-proportional grassland mixture receiving 150 N (high-diversity, low-input), and; (c) a 300 N *L. perenne* monoculture (low-diversity, high-input). Using predictions from the model (Eq. 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).

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). 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. 3a) and crop phase (Fig. 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.

4.5. *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 150 N *L. perenne* monoculture (low-diversity, low-input) as a reference level for yields in both the grassland and crop phases (Fig. 5), we compare the effect on yield for (a) the 150 N *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 150 N (high-diversity, low-input) to quantify the effect of an increase in plant diversity, and; (c) the 300 N *L. perenne* monoculture (low-diversity, high-input) to quantify the effect of an increase in fertiliser use.

Relative to the 150 N *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. 5a) than that from the equi-proportional 6-species mixture (Fig. 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. 5b), while higher fertiliser level (300 N *L. perenne*) resulted in lower yields (Fig. 5c).

Our results are in line with those of Eriksen (2001), who showed that an unfertilised 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. 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.

CRediT authorship contribution statement

GG, CB and **JF** conceived the ideas and designed the methodology; **GG** collected the data; **GG, CB** and **JF** analysed the data; **GG, CB** and **JF** led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

Data and statistical code is available from the Teagasc TStór repository at http://hdl.handle.net/11019/2801.

Acknowledgements

GG was funded by the Teagasc Walsh Scholarship Programme. CB was supported by the Science Foundation Ireland Frontiers for the Future Programme, grant number 19/FFP/6888.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2022.126531.

References

Bacon, P., 1995. Nitrogen Fertilization in the Environment. CRC Press.
Ball, B.C., Watson, C.A., Crichton, I., 2007. Nitrous oxide emissions, cereal growth, N recovery and soil nitrogen status after ploughing organically managed grass/clover swards. Soil Use Manage 23, 145–155. https://doi.org/10.1111/j.1475-2743.2006.00072.x.
Baxter, S., 2007. World reference base for soil resources. World Soil Resources Report. Food and Agriculture Organization of the United Nations, Rome, p. 103.
Birch, H., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10, 9–31. https://doi.org/10.1007/BF01343734.
Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biol. 15, 808–824. https://doi.org/10.1111/j.1365-2486.2008.01681.x.
Christensen, B.T., 1997. The Askov long-term field experiments. Arch. Agron. Soil Sci. 42, 265–278. https://doi.org/10.1080/03650349709385732.
Cornell, J.A., 2011. Experiments with Mixtures: Designs, Models, and the Analysis of Mixture Data. John Wiley & Sons.
Crotty, F.V., Fychan, R., Sanderson, R., Rhymes, J.R., Bourdin, F., Scullion, J., Marley, C. L., 2016. Understanding the legacy effect of previous forage crop and tillage management on soil biology, after conversion to an arable crop rotation. Soil Biol Biochem 103, 241–252. https://doi.org/10.1016/j.soilbio.2016.08.018.

Cummins, S., Finn, J.A., Richards, K.G., Lanigan, G.J., Grange, G., Brophy, C., Cardenas, L.M., Misselbrook, T.H., Reynolds, C.K., Krol, D.J., 2021. Beneficial effects of multi-species mixtures on N_2O emissions from intensively managed grassland swards. Sci Total Environ. 792, 148163 https://doi.org/10.1016/j.scitotenv.2021.148163.

Dardonville, M., Urruty, N., Bockstaller, C., Therond, O., 2020. Influence of diversity and intensification level on vulnerability, resilience and robustness of agricultural systems. Agric. Syst. 184, 102913 https://doi.org/10.1016/j.agsy.2020.102913.

Eisenhauer, N., Barnes, A.D., Cesarz, S., Craven, D., Ferlian, O., Gottschall, F., Hines, J., Sendek, A., Siebert, J., Thakur, M.P., Turke, M., 2016. Biodiversity-ecosystem function experiments reveal the mechanisms underlying the consequences of biodiversity change in real world ecosystems. J. Veg. Sci. 27, 1061–1070. https://doi.org/10.1111/jvs.12435.

Eisenhauer, N., Lanoue, A., Strecker, T., Scheu, S., Steinauer, K., Thakur, M.P., Mommer, L., 2017. Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. Sci. Rep. 7, 44641. https://doi.org/10.1038/srep44641.

Emadodin, I., Corral, D.E.F., Reinsch, T., Kluss, C., Taube, F., 2021. Climate change effects on temperate grassland and its implication for forage production: a case study from Northern Germany. Agriculture 11, 232. https://doi.org/10.3390/agriculture11030232.

Eriksen, J., 2001. Nitrate leaching and growth of cereal crops following cultivation of contrasting temporary grasslands. J. Agric. Sci. 136, 271–281. https://doi.org/10.1017/S0021859601008802.

European Commission, 2020. A farm to fork strategy: for a fair, healthy and environmentally-friendly food system. In: t.C., the European Economic and Social Committee and the Committee of the Regions (Ed.), Communication from the Commission to the European Parliament.

Finn, J.A., Kirwan, L., Connolly, J., Sebastia, M.T., Helgadottir, A., Baadshaug, O.H., Belanger, G., Black, A., Brophy, C., Collins, R.P., Cop, J., Dalmannsdottir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B.E., Ghesquiere, A., Golinska, B., Golinski, P., Grieu, P., Gustavsson, A.M., Hoglind, M., Huguenin-Elie, O., Jorgensen, M., Kadziuliene, Z., Kurki, P., Llurba, R., Lunnan, T., Porqueddu, C., Suter, M., Thumm, U., Luscher, A., 2013. Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: a 3-year continental-scale field experiment. J. Appl. Ecol. 50, 365–375. https://doi.org/10.1111/1365-2664.12041.

Fox, A., Luscher, A., Widmer, F., 2020a. Plant species identity drives soil microbial community structures that persist under a following crop. Ecol. Evol. 10, 8652–8668. https://doi.org/10.1002/ece3.6560.

Fox, A., Suter, M., Widmer, F., Lüscher, A., 2020b. Positive legacy effect of previous legume proportion in a ley on the performance of a following crop of Lolium multiflorum. Plant and Soil 447, 497–506. https://doi.org/10.1007/s11104-019-04403-4.

Franzluebbers, A.J., Sawchik, J., Taboada, M.A., 2014. Agronomic and environmental impacts of pasture-crop rotations in temperate North and South America. Agric. Ecosyst. Environ. 190, 18–26. https://doi.org/10.1016/j.agee.2013.09.017.

Garland, G., Edlinger, A., Banerjee, S., Degrune, F., Garcia-Palacios, P., Pescador, D.S., Herzog, C., Romdhane, S., Saghai, A., Spor, A., Wagg, C., Hallin, S., Maestre, F.T., Philippot, L., Rillig, M.C., van der Heijden, M.G.A., 2021. Crop cover is more important than rotational diversity for soil multifunctionality and cereal yields in European cropping systems. Nat. Food 2, 28–37. https://doi.org/10.1038/s43016-020-00210-8.

Götze, P., Rücknagel, J., Wensch-Dorendorf, M., Märländer, B., Christen, O., 2017. Crop rotation effects on yield, technological quality and yield stability of sugar beet after 45 trial years. Eur. J. Agron. 82, 50–59. https://doi.org/10.1016/j.eja.2016.10.003.

Grange, G., Finn, J.A., Brophy, C., 2021. Plant diversity enhanced yield and mitigated drought impacts in intensively managed grassland communities. J. Appl. Ecol. 58, 1864–1875. https://doi.org/10.1111/1365-2664.13894.

Guo, X.B., Drury, C.F., Yang, X.M., Reynolds, W.D., Fan, R.Q., 2014. The extent of soil drying and rewetting affects nitrous oxide emissions, denitrification, and nitrogen mineralization. Soil Sci. Soc. Am. J. 78, 194–204. https://doi.org/10.2136/sssaj2013.06.0219.

Hahn, C., Luscher, A., Ernst-Hasler, S., Suter, M., Kahmen, A., 2021. Timing of drought in the growing season and strong legacy effects determine the annual productivity of temperate grasses in a changing climate. Biogeosciences 18, 585–604. https://doi.org/10.5194/bg-18-585-2021.

Haughey, E., Suter, M., Hofer, D., Hoekstra, N.J., McElwain, J.C., Luscher, A., Finn, J.A., 2018. Higher species richness enhances yield stability in intensively managed grasslands with experimental disturbance. Sci. Rep. 8, 15047. https://doi.org/10.1038/s41598-018-33262-9.

Hegewald, H., Wensch-Dorendorf, M., Sieling, K., Christen, O., 2018. Impacts of break crops and crop rotations on oilseed rape productivity: a review. Eur. J. Agron. 101, 63–77. https://doi.org/10.1016/j.eja.2018.08.003.

Hofer, D., Suter, M., Haughey, E., Finn, J.A., Hoekstra, N.J., Buchmann, N., Luscher, A., 2016. Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. J. Appl. Ecol. 53, 1023–1034. https://doi.org/10.1111/1365-2664.12694.

Hossain, M.L., Li, J.F., 2020. Effects of long-term climatic variability and harvest frequency on grassland productivity across five ecoregions. Glob. Ecol. Conserv. 23, 1154. https://doi.org/10.1016/j.gecco.2020.e01154.

Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C., 2001. Climate Change 2001: the Scientific Basis. The Press Syndicate of the University of Cambridge.

Isbell, F.I., Polley, H.W., Wilsey, B.J., 2009. Species interaction mechanisms maintain grassland plant species diversity. Ecology 90, 1821–1830. https://doi.org/10.1890/08-0514.1.

Kirwan, L., Connolly, J., Finn, J.A., Brophy, C., Luscher, A., Nyfeler, D., Sebastia, M.T., 2009. Diversity-interaction modeling: estimating contributions of species identities and interactions to ecosystem function. Ecology 90, 2032–2038. https://doi.org/10.1890/08-1684.1.

Kollas, C., Kersebaum, K.C., Nendel, C., Manevski, K., Muller, C., Palosuo, T., Armas-Herrera, C.M., Beaudoin, N., Bindi, M., Charfeddine, M., Conradt, T., Constantin, J., Eitzinger, J., Ewert, F., Ferrise, R., Gaiser, T., de Cortazar-Atauri, I.G., Giglio, L., Hlavinka, P., Hoffmann, H., Hoffmann, M.P., Launay, M., Manderscheid, R., Mary, B., Mirschel, W., Moriondo, M., Olesen, J.E., Ozturk, I., Pacholski, A., Ripoche-Wachter, D., Roggero, P.P., Roncossek, S., Rotter, R.P., Ruget, F., Sharif, B., Trnka, M., Ventrella, D., Waha, K., Wegehenkel, M., Weigel, H.J., Wu, L.H., 2015. Crop rotation modelling-A European model intercomparison. Eur. J. Agron. 70, 98–111. https://doi.org/10.1016/j.eja.2015.06.007.

Leip, A., Billen, G., Garnier, J., Grizzetti, B., Lassaletta, L., Reis, S., Simpson, D., Sutton, M.A., de Vries, W., Weiss, F., Westhoek, H., 2015. Impacts of European livestock production: nitrogen, sulphur, phosphorus and greenhouse gas emissions, land-use, water eutrophication and biodiversity. Environ. Res. Lett. 10, 115004 https://doi.org/10.1088/1748-9326/10/11/115004.

Lemaire, G., Gastal, F., Franzluebbers, A., Chabbi, A., 2015. Grassland-cropping rotations: an avenue for agricultural diversification to reconcile high production with environmental quality. Environ. Manag. 56, 1065–1077. https://doi.org/10.1007/s00267-015-0561-6.

Lopez-Sangil, L., Hartley, I.P., Rovira, P., Casals, P., Sayer, E.J., 2018. Drying and rewetting conditions differentially affect the mineralization of fresh plant litter and extant soil organic matter. Soil Biol. Biochem. 124, 81–89. https://doi.org/10.1016/j.soilbio.2018.06.001.

Loreau, M., Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412, 72–76. https://doi.org/10.1038/35083573.

Lorenz, H., Reinsch, T., Kluß, C., Taube, F., Loges, R., 2020. Does the admixture of forage herbs affect the yield performance, yield stability and forage quality of a grass clover ley? Sustainability 12, 5842.

Mahmud, K., Panday, D., Mergoum, A., Missaoui, A., 2021. Nitrogen losses and potential mitigation strategies for a sustainable agroecosystem. Sustainability 13, 2400. https://doi.org/10.3390/su13042400.

Martin, G., Durand, J.-L., Duru, M., Gastal, F., Julier, B., Litrico, I., Louarn, G., Mediene, S., Moreau, D., Valentin-Morison, M., 2020. Role of ley pastures in tomorrow's cropping systems. A review. Agron. Sustain. Dev. 40.

Nemecek, T., Hayer, F., Bonnin, E., Carrouee, B., Schneider, A., Vivier, C., 2015. Designing eco-efficient crop rotations using life cycle assessment of crop combinations. Eur. J. Agron. 65, 40–51. https://doi.org/10.1016/j.eja.2015.01.005.

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., Luscher, A., 2009. Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. J. Appl. Ecol. 46, 683–691. https://doi.org/10.1111/j.1365-2664.2009.01653.x.

Rasmussen, J., Søegaard, K., Pirhofer-Walzl, K., Eriksen, J., 2012. N2-fixation and residual N effect of four legume species and four companion grass species. Eur. J. Agron. 36, 66–74. https://doi.org/10.1016/j.eja.2011.09.003.

Ravishankara, A.R., Daniel, J.S., Portmann, R.W., 2009. Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326, 123–125. https://doi.org/10.1126/science.1176985.

Reckling, M., Hecker, J.M., Bergkvist, G., Watson, C.A., Zander, P., Schlafke, N., Stoddard, F.L., Eory, V., Topp, C.F.E., Maire, J., Bachinger, J., 2016. A cropping system assessment framework-evaluating effects of introducing legumes into crop rotations. Eur. J. Agron. 76, 186–197. https://doi.org/10.1016/j.eja.2015.11.005.

Smith, R.G., Atwood, L.W., Warren, N.D., 2014. Increased productivity of a cover crop mixture is not associated with enhanced agroecosystem services. PLoS one 9, e97351. https://doi.org/10.1371/journal.pone.0097351.

Steenvoorden, J., Fonck, H., Oosterom, H., 1986. Losses of nitrogen from intensive grassland systems by leaching and surface runoff. Nitrogen Fluxes in Intensive Grassland Systems. Springer, pp. 85–97.

Van der Putten, W.H., Bradford, M.A., Pernilla Brinkman, E., van de Voorde, T.F., Veen, G., 2016. Where, when and how plant–soil feedback matters in a changing world. Funct. Ecol. 30, 1109–1121. https://doi.org/10.1111/1365-2435.12657.

Vogel, A., Scherer-Lorenzen, M., Weigelt, A., 2012. Grassland resistance and resilience after drought depends on management intensity and species richness. PLoS One 7, e36992. https://doi.org/10.1371/journal.pone.0036992.

Willems, A.B., Augustenborg, C.A., Hepp, S., Lanigan, G., Hochstrasser, T., Kammann, C., Muller, C., 2011. Carbon dioxide emissions from spring ploughing of grassland in Ireland. Agric. Ecosyst. Environ. 144, 347–351. https://doi.org/10.1016/j.agee.2011.10.001.