RESEARCH ARTICLE

THE ROLE OF LONG-TERM EXPERIMENTS IN VALIDATING TRAIT-BASED APPROACHES TO ACHIEVING MULTIFUNCTIONALITY IN GRASSLANDS

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HIGHLIGHTS

- Data from the Park Grass Experiment shows inherent trade-offs between species richness, biomass production and soil organic carbon.
- Soil organic carbon is positively correlated with biomass production that increases with fertilizer additions.
- Variance in outcomes can be understood in terms of the dominant ecological strategies of the plant communities indicated by functional traits.
- There was an indication that data on traits associated with the spatiotemporal pattern of resource capture could be used to design species mixtures with greater resource use complementarity, increasing species richness without sacrificing productivity.
- Variance in soil organic carbon was positively correlated with pH.

GRAPHICAL ABSTRACT
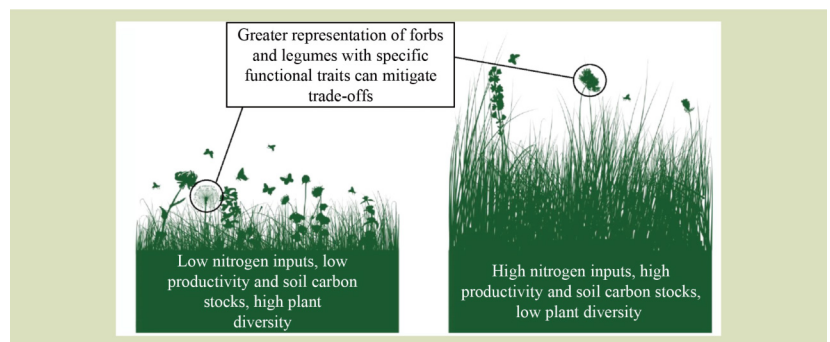
ABSTRACT

Quantifying the relationships between plant functional traits and ecosystem services has been promoted as an approach to achieving multifunctional grassland systems that balance productivity with other regulating, supporting and cultural services. Establishing trade-offs and synergies between traits and services has largely relied on meta-analyses of studies from different systems and environments. This study demonstrated the value of focused studies of long-term experiments in grassland systems that measure traits and services in the same space and time to better understand the ecological constraints underlying these trade-offs and synergies. An analysis is presented that uses data from the Park Grass Experiment at Rothamsted Research on above-ground productivity, species richness and soil organic carbon stocks to quantify the relationships between these three outcomes and the power of variance in plant functional traits in explaining them. There was a trade-off between plots with high productivity, nitrogen inputs and soil organic carbon and plots with high species richness that was explained by a functional gradient of traits that are indicative of contrasting strategies of resource acquisition of resource conservation. Examples were identified of using functional traits to identify opportunities for mitigating these trade-offs and moving toward more multifunctional systems.

1 INTRODUCTION

The conversion of semi-natural grasslands and extensively managed hay meadows in Europe to arable production or grasslands managed for intensive silage or livestock production has focused attention on the other ecosystem services (ES) that grasslands deliver for human wellbeing. The Millennium Ecosystem Assessment defined ES as "the benefits people obtain from ecosystems" and identified four categories: provisioning, regulating, supporting and cultural services[1]. In the context of the intensification of European grasslands, the drive for productivity (a provisioning service) has been at the expense of greenhouse gas (GHG) emissions and carbon sequestration (regulating services), soil health (supporting service) and biodiversity (cultural and regulating service)[2,3].

The contribution of the livestock sector to all anthropogenic GHG emissions has been estimated at 14.5%[4]. Although there are opportunities to mitigate emissions through targeted livestock management[5], the intrinsic potential of permanent grasslands to sequester carbon could also be enhanced to offset the negative impact of grazing. Although the rate of sequestration of a specific grassland will be determined by management, soil and climate interactions, a meta-analysis reported a potential C sequestration rate for grasslands of 0.22 Mg·ha^{-1}·yr^{-1} C[6]. The increased use of mineral fertilizers on grasslands has also made a significant contribution to GHG emissions (through emissions of nitrogen oxides and use of fossil fuels in their manufacture) and also has the potential to compromise soil function through altering the soil chemical/biological/physical phenotype[7] and lead to decreases in plant diversity through eutrophication[8]. Where this results in a loss of flowering plants, intensification of grassland is also a contributing factor to pollinator declines and the regulating service they provide to agriculture[9]. Providing the regulating, supporting and cultural services provided by biodiversity while maintaining the provisioning service of primary production in reduced input systems remains a major challenge for agricultural science[3].

One approach to quantifying the relative delivery of multiple ES of a grassland or a landscape, and potentially reconciling production with regulating, supporting and cultural services, is to characterize the sward in terms of the plant functional traits, for example vegetative height or leaf traits such as leaf dry matter content[10]. These traits will be indicative of both the processes underlying the assembly of plant communities in *response* to management and environment and the effect of the community in terms of their ecosystem function and delivery

of ES[11]. Conceptual frameworks that explore the relative contribution of trait functional diversity and/or community weighted means (CWM) of individual traits are now well established in the literature[12] and have been used to explore relationships between plant traits and ES across multiple habitats and systems[13]. A recent meta-analysis of published trait-ES relationships in grassland identified trade-offs and synergies and trait-service groups that were indicative of intrinsic eco-evolutionary constraints that determine ecosystem functions[14]. For example, a suite of root traits was associated with erosion control and water regulation and leaf traits with fodder quality. An understanding of the implications of a change in the functional composition of a sward for multiple ES would facilitate the management of grasslands that targeted specific ES or attempted to reconcile multiple ES[15].

Analyses of trade-offs and synergies between ES delivered by grasslands and relationships with functional traits have largely been based on comparisons of multiple studies across contrasting environments[3,14,16]. Evidence is, therefore, correlative with limited power to establish mechanistic links between traits and services. Where comparisons have been done on a single site, additional care needs to be taken to control for legacies for contrasting management or transitional community dynamics[17]. Long-term experiments (LTEs) on grassland systems are potentially a unique resource for overcoming these limitations; management is controlled and plant communities are assumed to be in equilibrium with their local environment, avoiding the problem of transitional dynamics[18]. LTEs have been used to quantify ES individually, for example, productivity and biodiversity[19] and carbon sequestration[20], however, their potential for validating the trait-based approach to achieving multifunctionality in grassland systems has yet to be fully realized.

In this study, we demonstrate the opportunity LTEs offer for studying multiple ES from the same location and at the same time by analyzing data from the Park Grass Experiment (PGE) at Rothamsted Research (UK) on above-ground productivity, plant diversity and soil organic carbon (SOC). While these variables can be understood as surrogates for provisioning, cultural and regulating services, respectively, it could be argued that they are not all in themselves, ES. Therefore, here, we describe them as outcomes. We explore: (1) the relationships between the three outcomes and (2) the potential for functional traits to explain variance in the outcomes and to inform strategies to optimize multifunctionality particularly where there are trade-offs between outcomes.

2 MATERIALS AND METHODS

2.1 The Park Grass Experiment (PGE)

The PGE is located at Rothamsted Research, Harpenden, Hertfordshire, UK (51°48′ N, 0°22′ W) on 2.8 ha of moderately well drained silty clay loam overlying clay-with-flints. When the experiment began in 1856, the soil pH was 5.4–5.6 and the nutrient status was poor. The field was in permanent pasture for at least 100 years prior to 1856 and the original classification of the vegetation was dicotyledon-rich *Cynosurus cristatus-Centaurea nigra* grassland[21]. The experiment was established to compare the effects of different amounts and combinations of fertilizers (both organic and mineral) on hay yields. The plots have been cut in mid-June and made into hay; for 19 years, the regrowth was grazed by sheep penned to individual plots, but since 1875 a second cut, usually carted green, has been taken. The original treatments included controls (no inputs) and various combinations of phosphorus, potassium, magnesium and sodium with nitrogen applied at increasing rates either as sodium nitrate or ammonium sulfate (Fig. 1). Detailed plans and descriptions of the design of the PGE over time along with associated metadata can be obtained from the Rothamsted Electronic Archive. Three plots have received or continue to receive organic amendments, either as farmyard manure (FYM) or fishmeal or poultry manure. The application of N as ammonium sulfate has had an acidifying effect on the soil, reducing pH to as low as 3.6 in some plots. To account for this confounding effect on yields, the experiment was split in 1903 and lime was added to half of most of the main plots and in 1965 the plots were further divided to give the four subplots (a to d) that have continued since. Three subplots receive lime every three years, if necessary, to maintain a target soil pH of 7, 6 and 5 for subplots a, b and c, respectively. The final subplot (d) was left unlimed. The PGE predates the development of modern statistics and has no formal replication or blocking design discounting traditional factorial statistical analyses. However, the contrasting fertilizer treatments and age of the experiment have resulted in long spatiotemporal gradients for multiple variables that can be analyzed using regression techniques.

We selected a 5-year period over which all three outcomes were measured (1998–2002). Above-ground biomass was recorded every year; data on species separations of all plots were available for 1998, 1999 and 2000, and SOC was measured in the top 23 cm in 2002. Finally, soil pH was measured in 1998 and 2002. To covert %SOC to carbon stocks ha^{-1}, data on bulk density was used. Soil weights were measured outside the study period (2011–2012) but have changed little over time with only small differences between the plots[22]. Recent soil weight data were not available for all subplots, where data were missing the value for the closest plot in terms of fertilizer treatment were used. Biomass and species composition on the PGE is known to respond to short-term perturbation[23] and mean values over the selected time period for these outcomes were used in the analysis. During this period, the PGE had 97 subplots (Fig. S1). Subplots that receive N as ammonium sulfate with no additional lime now have a soil pH of 3.6−4.1 (compared to an original pH for the field of 5.3); this abiotic stress has resulted in atypical dynamics and extreme outcomes in terms of species richness[19] and SOC (these plots have a thick layerof undecomposed dead plant material on the surface)[24]. As these plots are not typical of improved acid grassland, they were not included in the analysis. Between 1989 and 1996, treatments were also changed on three main plots and hence the corresponding 12 subplots were also excluded from the analysis. The sum of these exclusions left a total of 74 subplots to study.

2.2 Hay yield and above-ground biomass

During the period of study here (1998–2002), hay yield and above-ground biomass were measured from a yield strip of known area using a tractor-powered forage harvester with a 1.1-m wide cut and collection box (John Wilder Ltd., Wallingford, UK). For the wider subplots in the PGE, two yield strips were cut and combined, whereas only one yield strip was cut for the narrower-width subplots. Grass collected in the box from each subplot was raked onto a net and weighed directly in the field on frame-mounted scales, before a subsample was taken for dry matter determination and other analyses. The yield strip was moved each year to avoid consecutive yield measurements being made on exactly the same part of the plots.

2.3 Soil organic carbon

In autumn 2002 topsoil (0–23 cm) on all subplots were sampled by hand using a gouge auger (2 cm diameter); 12–20 cores were taken per subplot and bulked, depending on the plot area. The soils were broken up by hand and laid out to air-dry at room temperature for 7–10 days, after removing vegetation, stones and soil fauna (e.g., worms). Air-dry soils were milled to < 2 mm and a subsample was fine-milled (< 44 mesh; < 360 μm) prior to analysis of total C using a LECO TruMac Combustion Analyserand inorganic C by manometry. Total soil organic C (%SOC) was determined by the difference between total C and inorganic C. Topsoil weights (Mg·ha^{-1} oven dry soil) were determined for 10 plots between 2011 and 2012. Three intact

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soil cores (5 cm × 23 cm), were collected from each subplot (total of 12 cores per main plot) using a mechanical soil sampler to calculate bulk density (g·cm⁻³ soil). The total fresh weight of the cores was determined before sieving to remove stones, vegetation and soil fauna. Subsamples of sieved soil were dried at 105 °C overnight to determine the soil dry matter content.

2.4 Species richness and plant community composition

The data for species richness and proportional biomass of plant species and functional types were obtained from the 10 year vegetation survey completed between 1991 and 2000[19]. For each year of the study period, six randomly located quadrats measuring 50 cm × 25 cm were located within each subplot in early June. The herbage was cut with scissors at ground level and the fresh material sorted to species in the laboratory before oven drying at 80 °C for 24 h to determine the dry mass for each species. A figure of cumulative species richness was drawn from the 3 years of data used in this study (1998–2000) for each subplot. The data on proportional biomass was used to calculate the values for CWM of selected functional traits (Eq. (1)).

$$\text{CWM} = \sum_{i=1}^{n} p_i \times \text{trait}_i \qquad (1)$$

where, n is the number of species in community, p_i is the proportion of species i (weight of species i/total weight) and trait$_i$ is the trait value for species i.

Traits included in the analysis were derived from online databases and were, therefore constrained by the availability of data for the species pool on the experiment. Life form, month of first flowering, duration of flowering (in months) and maximum vegetative height were obtained from the UK Ecoflora database[25], leaf dry matter content (LDMC) and specific leaf area (SLA) from the LEDA trait database[26], biomass allocation above and below ground from Bergmann et al.[27] and seed weight from the seed database held at Kew Gardens, UK[28]. Relationships between traits were visualized using a principal components analysis (PCA). A full species list with trait values can be found in Table S1.

2.5 Statistical analysis

Simple linear regression analysis was used to determine the relationships between the three outcomes and the relationship between inputs of inorganic N and SOC. Two trade-offs were identified from this analysis: plots with higher productivity had reduced species richness and greater SOC stocks were observed on plots with greater N inputs (associated with a greater global warming potential (GWP) because of emissions of nitrogen oxides from the fertilizer and use of fossil fuels in their manufacture). To identify plots that had positive residuals, potentially mitigating these trade-offs, two further response variables were derived: species richness adjusted for above-ground biomass and SOC adjusted for N inputs.

To quantify the relative contribution of abiotic drivers and the value of incorporating data on plant functional traits in explaining variance in the five response variables, a similar analytic framework to that proposed by Diaz et al.[12] was followed. A series of generalized linear models (GLM; using an identity link function for all response variables) were compared. In the first step, the combined effects of all abiotic factors (amount of added nitrogen, +/− P, +/− K, +/− organic amendments, pH) were tested. Secondly, the effects of the CWM of individual traits were analyzed and colinearity between traits quantified in a correlation matrix. For each of the five response variables, all subsets multiple linear regression was then also used to quantify the additional explanatory power of incorporating plant functional trait data into models that include the effects of abiotic factors. As opposed to a stepwise approach, all subsets regression analyses all possible combinations of explanatory variables using the adjusted r^2 and Mallows C_p as criteria for comparing models and selecting the parsimonious number of covariates to include. For each response variable, the abiotic factors selected from the first step were included in all models and the most parsimonious model selected that included significant effects of CWM traits, only including independent CWM traits. Diaz et al. propose an additional step to capture idiosyncratic effects of individual species; in our system, we expected the response variables to be sensitive to the relative facilitation effect of legumes and, therefore included the proportion of legumes as a term in the model selection. Finally, the effect of functional diversity was analyzed by adding the Rao coefficient for each individual trait as model terms in the all subsets GLM but only including the significant abiotic factors and CWM traits that had been selected in the previous step.

3 RESULTS

In the PGE, plots lacking P and/or N have the smallest above-ground productivity and the greatest proportion of forbs. The plots with P and K added but without (or at low rates of) N support the greatest proportion of legumes and have intermediate productivity. The most productive plots have the

greatest rate of N applied (at 144 kg·ha⁻¹ N) together with the other macronutrients and are dominated by grasses (Fig. 1).

Over the time period analyzed, there was a strongly negative relationship between above-ground productivity and species richness and a positive relationship between above-ground productivity and SOC stocks. (Fig. 2(a,b)). Consequently, species richness was also significantly negatively related to SOC (Fig. 2(c)). The response of above-ground productivity to increasing inputs of N was also reflected in a strong positive relationship of SOC with N inputs (Fig. 2(d)).

The PCA of plant functional traits represented by the species pool in the PGE reflected broad plant ecological strategies (PES)[29] (Fig. 3). The first axis discriminated between traits associated with a ruderal strategy (short life span, high SLA and early flowering) and the second axis between traits associated

with competition for light (tall stature) and resource conservation (or tolerance of stress (high LDMC, long life span)). Many traits were colinear reflecting the fact that they covary and are indicative of underlying ecophysiological trade-offs (Table S2).

When the effects of individual traits on the five response variables was analyzed, variance in all outcomes could be partly explained by multiple traits (Table 1). These relationships were largely consistent with the contrasts in PES discussed above. Productivity (total biomass) was higher on plots characterized by traits associated with resource acquisition (> CWM SLA) and competition for light (> CWM height, > CWM allocation) and had higher proportions of shorter-lived species with earlier flowering. In our data, variance in SOC appears to be driven by carbon inputs from plant production with a positive relationship between SOC and above-ground productivity (Fig. 2(b)). Consequently, a similar suite of traits explained variance in SOC as total biomass.

Plots with higher species richness were associated with traits that are more indicative of a stress-tolerant, resource conservation strategy (> CWM life span) and competition for below-ground resources (< CWM height and < CWM allocation). There were fewer significant effects of individual traits on species richness adjusted for biomass and SOC adjusted for N inputs. However, plots that supported more species than would be expected from the relationship with biomass appear to be characterized by a mix of traits that balance competition for above- and below-ground resources (< CWM height but > CWM allocation).

Although there were significant relationships with CWM lateral spread and CWM Ln seed mass with SOC adjusted for N inputs, functional traits were not retained in the optimal model for either SOC or adjusted SOC; variance was explained best by N inputs and pH; importantly, the addition of organic amendments was not a significant term in the models. However, incorporating plant functional effects did improve the explanatory power of the models for the remaining outcomes (Table 2). SLA was retained in the model of total biomass, allocation in species richness and LDMC in the model for species richness adjusted for biomass (Fig. 4). In this last model, functional diversity of LDMC was also retained in the model.
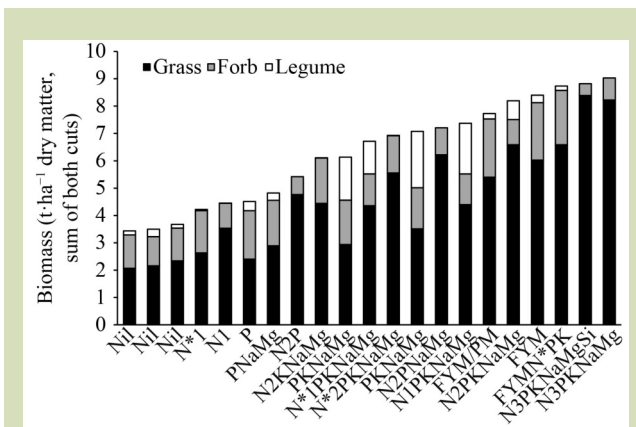

Fig. 1 Effect of fertilizer treatments on above-ground productivity (sum of both cuts averaged for harvest years 1998–2002). Data are presented at the level of the main plot, averaging biomass across the subplots and omitting the strongly acid subplots with a pH of < 4.5. Three main plots did not receive any fertilizer inputs (Nil). Nitrogen was applied at increasing rates (48, 96 and 144 kg·ha⁻¹ N for N1, N2 and N3, respectively) either as ammonium sulfate (N) or sodium nitrate (N*). When applied as sodium nitrate, plots also received an additional 78 and 157 kg·ha⁻¹ S at N*1 and N*2, respectively. Phosphorus was applied at 35 kg·ha⁻¹ P as triple superphosphate, potassium at 225 kg·ha⁻¹ K as potassium sulfate, sodium at 15 kg·ha⁻¹ Na as sodium sulfate (also supplying 10 kg·ha⁻¹ S), magnesium at 10 kg·ha⁻¹ Mg as magnesium sulfate (also supplying 13 kg·ha⁻¹ S) and silicon at 135 kg·ha⁻¹ Si as water soluble sodium silicate (also supplying 63 kg·ha⁻¹ S). Organics were applied every fourth year to plots receiving farmyard manure (FYM) at 35 t·ha⁻¹ and pelleted poultry manure (PM) supplying approximately 240 and 65 kg·ha⁻¹ N, respectively.

4   DISCUSSION

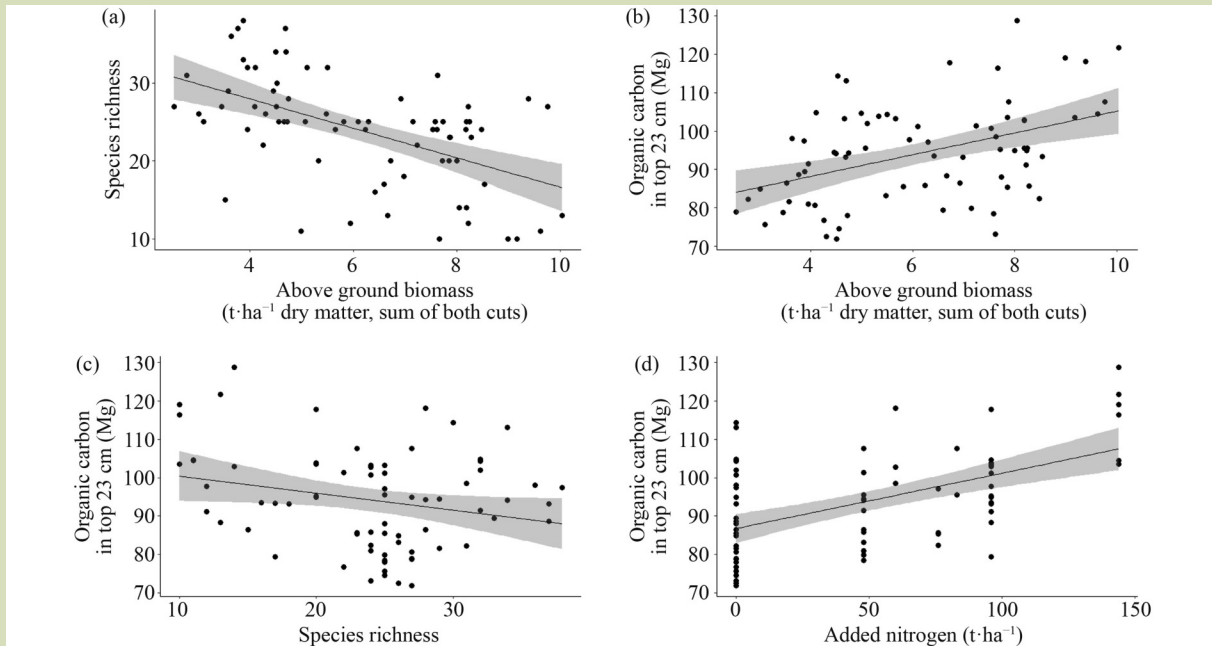A trait-based approach to the assessment of ES delivered by

Fig. 2   Relationships between outcomes (a–c) and trade-off between soil organic carbon (SOC) and inorganic nitrogen (N) inputs (d) with 95% confidence intervals. There is a negative relationship between above-ground biomass and species richness (species richness = 35.5 − 1.89 × biomass, %var = 27.7, F prob. < 0.001) and species richness and SOC (SOC = 105 − 0.5 × species richness, %var = 4.6, F prob. = 0.04) and a positive relationship between SOC and above-ground biomass (SOC = 76.7 + 2.83 × biomass, %var = 17.9, F prob. < 0.001) and SOC and added N (SOC = 86.7 + 0.14 × added N, %var = 27.5, F prob. < 0.001).
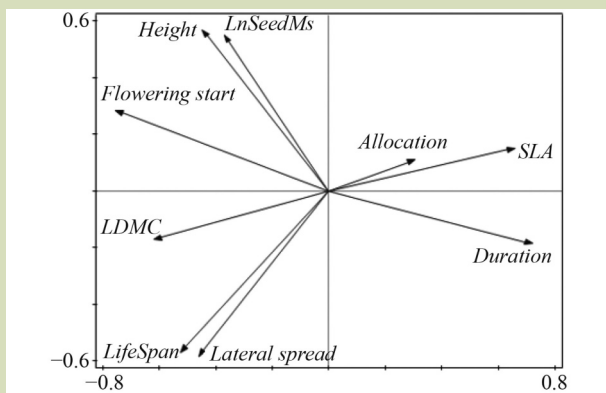

Fig. 3   Principal component analysis of traits represented by the plant species in the Park Grass Experiment.

grassland systems has the potential to both provide a framework for managing grasslands to deliver single or multiple ES[12] and further our understanding of the ecological processes that underlie trade-offs and synergies between outcomes[30]. When considering the three outcomes included in this analysis (above-ground productivity, species richness and SOC) intrinsic properties of the grassland system in the PGE plots mean that there are inevitable trade-offs and no

single management treatment maximizes all three ES. The multivariate analysis of the relationships between the functional traits represented by the plant communities in the PGE confirmed axes of plant strategies that reflect trade-offs between resource acquisition and conservation[18] and can be used to explain the contrasting response of the outcomes.

The trade-off between above-ground biomass and species richness in the PGE has been reported by Crawley et al.[19] and discussed in the context of the biotic and abiotic drivers of community assembly. In our study, by excluding the acid plots that limit the persistence of species to those that are tolerant of the abiotic stress of pH, we found a much stronger negative relationship between these two outcomes (with an r^2 of 0.43 compared to 0.05 in the Crawley study). This is indicative of the ecological process of competitive exclusion being the main driver of the observed relationship; the addition of inorganic fertilizers has effectively made below-ground resources non-limiting and hence selected for plant species that are adapted for above-ground competition (rapid growth preempting space and tall stature shading out more stress-tolerant and prostrate species). Height was positively related to above-ground biomass and negatively with species richness and allocation to roots had the opposite effect, confirming the role of

Table 1 Effect of individual community weighted means traits on outcomes (SOC = soil organic carbon) on the Park Grass Experiment indicating direction of response and level of significance

Item	Height	Lat.	SLA	LDMC	Allo.	Life	Seed	Flower	Dur.
Biomass	+***	−***	+***	+***	+***	−***	+*	−***	+***
Species richness	−***	ns	−**	−***	−***	+*	ns	+***	+*
SOC	+**	−**	ns	ns	+**	−**	+*	−*	ns
Adjusted species richness	+***	−**	ns	−***	−***	ns	ns	ns	+***
Adjusted SOC	ns	−*	ns	ns	ns	ns	+**	ns	ns

Note: + , significant positive effect; -, significant negative effect; ns, no significant effect; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$; Lat., lateral spread; Allo., allocation (above-ground/below-ground biomass); and Dur., flowering duration. For full description of traits see Table S1.

Table 2 Best predictive model combining abiotic factors and functional trait data for outcomes on the Park Grass Experiment

Item	%var	Function
Biomass	78.9	$y = -6.20 + 0.023 \times N + 1.17 \times P + 7.17 \times legumes + 0.39 \times SLA$
Species richness	77.4	$y = 40.81 - 0.07 \times N + 2.12 \times pH - 62.2 \times allocation$
SOC	43.0	$y = 44.79 + 0.13 \times N + 7.14 \times pH$
Adjusted species richness	58.2	$y = 43.6 - 0.06 \times N + 5.78 \times K + 2.4 \times pH - 0.16 \times LDMC - 15.62 \times FD\_LDMC$
Adjusted SOC	21.2	$y = -42.39 + 7.09 \times pH$

Note: Phosphorus and potassium were included in the model as factors (+/−) and model parameters are, therefore, differences compared to the reference level (minus these nutrients). SOC = soil organic carbon, SLA = specific leaf area, LDMC = leaf dry matter content.

competition for light in driving community assembly. The collinearity of traits means that the particular trait retained in the final model should be interpreted as being indicative of these contrasting plant strategies; in the case of total biomass, a high SLA (retained in the model of total biomass) is associated with an adaptation for resource acquisition in fertile environments[31] and allocation (retained in the model of species richness) is indicative of below-ground competition and a more stress-tolerant community.

Of particular interest are the plots that potentially mitigate this negative trade-off between biomass and species richness in the PGE, i.e., which support greater species richness for a given level of productivity. By studying these plots, new insights into the potential role of multispecies swards in supporting sustainable grassland systems can be gained[32]. Analyzing the residuals from the regression of species richness and above-ground productivity, plots with positive residuals were characterized by a mixture of traits related to resource use in space and time (for example, plant height, lateral spread and flowering duration) indicating the increased resource use complementarity on these plots. Adjusted species richness was also the only response variable that retained a measure of functional diversity in the model again supporting the

hypothesis that these plots supported a diversity of PES potentially through greater evenness of resource ratios[33]. The competitive dynamics on these plots warrants further investigation; the forb species that are driving the relationship with adjusted species richness appear to differ from those that are most susceptible to competitive exclusion and include *Plantago lanceolata* and *Ranunculus acris* (Fig. S2). As opposed to being subordinate, unproductive species that are characteristic of low fertility plots, these more competitive forbs appear to be making a significant contribution to above-ground productivity. It is likely that the partial reconciliation of the trade-off between productivity and diversity on these plots is related to the ratio of nutrients in the soil (determining niche partitioning)[33] which should be a focus of further work in the PGE.

The amount of SOC in the top 23 cm of the PGE plots measured in 2002 was strongly related to C inputs from plant production; the addition of organic amendments was not found to be a significant factor in the models. This is in contrast to a recent study on selected PGE plots that found no relationship between SOC and above-ground plant biomass[24]. This can be explained by the fact that we excluded the acid plots that we argue are atypical because of the abiotic stress
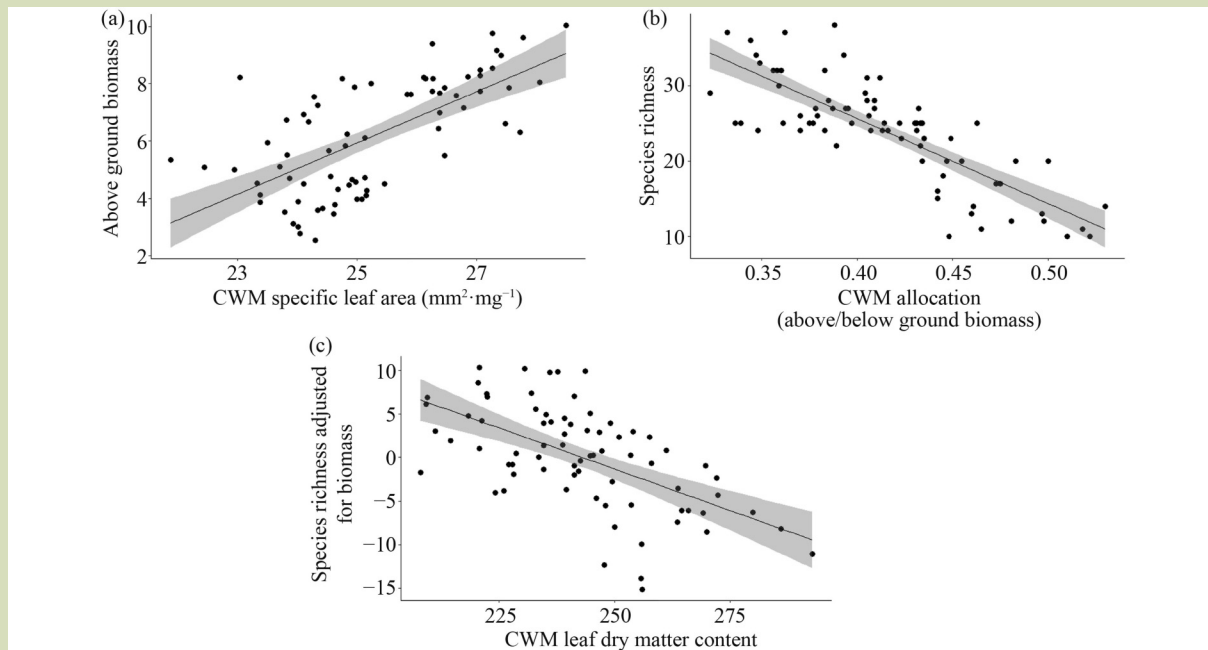
Fig. 4 Significant traits retained in most parsimonious model explaining variation in outcomes of the Park Grass Experiment, with 95% confidence intervals, using a combination of abiotic factors and functional traits, (a) relationship between community weighted means (CWM) specific leaf area and total biomass, (b) CWM allocation and species richness and (c) CWM leaf dry matter content and species richness adjusted for biomass.

associated with low pH and the larger number of plots we included in our study. Fornara et al.[24] reported the positive effect of liming on SOC stocks in the PGE and attributed this to greater biological activity in the limed soils leading to plant C inputs being processed and incorporated into resistant soil organomineral pools[24]. The dominant effect of abiotic drivers in explaining the variance in SOC between the PGE plots was confirmed by our analysis, which indicated that adding plant functional diversity effects did not improve the explanatory power of a model that only retained N inputs and pH (Fig. S3). This is despite the fact that we would expect traits associated with growth rate, life span and residue composition to be important in determining carbon dynamics[34]. This may be partly explained by the lack of data on traits that may be important in explaining this outcome (including C:N ratios) but also highlights an important limitation of our study and potentially of trait-based approaches to ES assessment. Here, we assume that variation in outcomes can be explained by functional contrasts in the plant community resulting from the different fertilizer treatments in the experiment. This ignores the potential role of interactions between management and other parts of the system (for example soil microbiology) in determining variation in the outcomes. Future work should integrate the indirect effect of variation in plant functional composition with data on soil properties and microbiology to

partition variance between different parts of the system and better understand the drivers of C sequestration in grassland[17].

By analyzing multiple outcomes of the PGE, our intention was not to fully explain the ecosystem processes determining variance in any one outcome but to illustrate the potential for LTEs to be used in combination with trait-based approaches to ES assessment. In so doing we have identified inherent constraints and trade-offs, opportunities for improving multifunctionality and generated hypotheses that can be challenged in future work. We see particular merit in focusing on plant communities that mitigate trade-offs and understanding the ecological mechanisms underlying potential synergies. We also see great potential in applying this approach in a more thorough study of the PGE, in combination with other grassland LTEs, that includes a wider range of ES and coordinates measurements in time using the same protocols. Such a study would provide an opportunity to overcome several weaknesses of the current study. First, the choice of traits to include in the analysis was constrained by the availability of data in online databases and did not include important traits that we would expect to explain variation in the outcomes, including leaf C:N ratio and root depth. Secondly, it is likely that there is large intraspecific variation in

traits between the plots in the PGE[16] and it would be beneficial to measure traits directly as opposed to relying on database values.

5   CONCLUSIONS

Long-term experiments, like the PGE, were set up to address specific hypotheses to do with the response of the variable of interest to a range of treatments; in the case of the PGE, the effect of fertilizers on hay yield. However, they are now unique platforms for studying the interaction of multiple response variables that make them an important resource in the drive for the sustainable intensification of agricultural systems. The results presented in this study illustrate the trade-offs and synergies between three outcomes and the potential for functional traits to be used as a common metric for modeling the behavior of the system and identifying opportunities to improve multifunctionality in managed grasslands. However, our analysis relied on the serendipitous collection of data on the three outcomes over the same time period. In this sense, the sampling campaigns were not planned with questions of multifunctionality in mind. While this study serves as a useful proof of concept, therefore, there is potential to use this modeling framework in the future as a template for designing coordinated sampling campaigns of a common set of response variables and using standardized protocols across multiple long-term experiments.

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