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Elevated CO_2 interacts with nutrient inputs to restructure plant communities in phosphorus-limited grasslands

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Abstract

Globally pervasive increases in atmospheric CO_2 and nitrogen (N) deposition could have substantial effects on plant communities, either directly or mediated by their interactions with soil nutrient limitation. While the direct consequences of N enrichment on plant communities are well documented, potential interactions with rising CO_2 and globally widespread phosphorus (P) limitation remain poorly understood. We investigated the consequences of simultaneous elevated CO_2 (eCO_2) and N and P additions on grassland biodiversity, community and functional composition in P-limited grasslands. We exposed soil-turf monoliths from limestone and acidic grasslands that have received >25 years of N additions (3.5 and 14 g m^{-2} year^{-1}) and 11 (limestone) or 25 (acidic) years of P additions (3.5 g m^{-2} year^{-1}) to eCO_2 (600 ppm) for 3 years. Across both grasslands, eCO_2, N and P additions significantly changed community composition. Limestone communities were more responsive to eCO_2 and saw significant functional shifts resulting from eCO_2–nutrient interactions. Here, legume cover tripled in response to combined eCO_2 and P additions, and combined eCO_2 and N treatments shifted functional dominance from grasses to sedges. We suggest that eCO_2 may disproportionately benefit P acquisition by sedges by subsidising the carbon cost of locally intense root exudation at the expense of co-occurring grasses. In contrast, the functional composition of the acidic grassland was insensitive to eCO_2 and its interactions with nutrient additions. Greater diversity of P-acquisition strategies in the limestone grassland, combined with a more functionally even and diverse community, may contribute to the stronger responses compared to the acidic grassland. Our work suggests we may see large changes in the composition and biodiversity of P-limited grasslands in response to eCO_2 and its interactions with nutrient loading, particularly where these contain a high diversity of P-acquisition strategies or developmentally young soils with sufficient bioavailable mineral P.

KEYWORDS
elevated CO_2, grasslands, nitrogen deposition, phosphorus limitation, plant communities

1 | INTRODUCTION

Anthropogenic activities have doubled the global cycling of N (Fowler et al., 2013) and raised the atmospheric concentration of CO_2 to over 417 ppm—a level not experienced in the past three million years of land plant evolution (Willeit et al., 2019). The productivity of many ecosystems globally is limited or co-limited by P availability, with recent estimates suggesting that up to 40% of the land's surface could be P limited in some way (Du et al., 2020). Atmospheric N deposition arising from agricultural intensification and fossil fuel combustion is likely to increase the prevalence and severity of P-limited ecosystems, yet we know comparatively little about how P-limited systems respond to concurrent global change drivers such as elevated CO_2 and N deposition.

Grasslands are the most extensive terrestrial ecosystem on Earth (Ali et al., 2016), covering almost a third of the land's surface (Latham et al., 2014), and are the most widespread P-limited ecosystems in temperate regions (Watson et al., 2011). They may represent up to a fifth of terrestrial net primary productivity (Chapin et al., 2011) and provide numerous ecosystem services, including storing an estimated 343 Pg C in the top 1 m of soil (Conant et al., 2017), as well as reducing leaching of nutrient pollution to watercourses (Phoenix et al., 2003). Moreover, species-rich grasslands are among the most biodiverse habitats in temperate regions (Carbutt et al., 2017), with almost one in five of Europe's endemic vascular plant species being found exclusively in grasslands (Habel et al., 2013), including many scarce and threatened plant species (Stroh et al., 2014). Grassland biodiversity is integral to the provisioning of ecosystem services (Isbell et al., 2011), yet species-rich temperate grasslands are one of the most threatened biomes on Earth (Hoekstra et al., 2004).

Plant species richness is typically reduced when soil nutrient availability is increased (Ceulemans et al., 2014; Harpole et al., 2016; Lambers et al., 2008), with the most diverse communities occurring disproportionally in highly weathered P-limited soils (Laliberté et al., 2013; Lambers et al., 2013). Over millennia, P limitation has facilitated the evolution of multiple specialist plant strategies to deploy photosynthate to acquire P from its diverse organic and mineral forms in soil (Ceulemans et al., 2017; Phoenix et al., 2020; Vance et al., 2003). These include the secretion of organic acids (Shane et al., 2006) and phosphatase enzymes (Margalef et al., 2017), investment in mycorrhizal fungal partners (Smith & Read, 2008) and increasing root surface area (Li et al., 2017). Such specialisation of plant root traits can reduce interspecific competition for P by creating different P niches, enabling the assembly and maintenance of highly diverse plant communities (Ceulemans et al., 2017; Phoenix et al., 2020). Alteration of nutrient and photosynthate availability, such as with nutrient pollution and eCO_2, respectively, has the potential to decouple these relationships, alter plant community composition and reduce biodiversity.

The consequences of increased N availability to terrestrial ecosystems, including grasslands, have been well studied and frequently show that plant diversity is adversely affected by N deposition (Bobbink et al., 2010; Phoenix et al., 2012; Stevens et al., 2004,

2010), leading to dominance by graminoids at the expense of legumes and other forbs (Lu et al., 2021). Indeed, N deposition has been identified as the third greatest threat to biodiversity globally (Sala et al., 2000). In N-limited ecosystems, N deposition may lead to rapid growth of the most nitrophilous species, competitively excluding more sensitive and slower-growing species (Bobbink et al., 2010). However, where P limitation constrains plant productivity, increases in N deposition may not promote growth and competitive exclusion unless they facilitate higher rates of soil organic P mineralisation [for example through greater N investment in enzymes (Chen et al., 2020)] or greater weathering of mineral P [such as apatite, via soil acidification (Blum et al., 2002)].

By comparison, we know less about the potential consequences of eCO_2 on plant communities, especially when it occurs in parallel with increases in nutrient availability. We might expect diverse plant communities to be more sensitive to eCO_2-induced shifts in community composition than their species-poor counterparts (He et al., 2002), as a larger species pool increases the likelihood of it containing CO_2-responsive species (Niklaus et al., 2001). Responses of plant communities to eCO_2 can also depend on a range of factors, such as plant species and functional type (Hanley et al., 2004; Poorter & Navas, 2003; Stöcklin et al., 1998), plant diversity (He et al., 2002), soil N availability (Reich et al., 2018) and the interactions between these (Reich, 2009; Zhu et al., 2020). For instance, the growing season of dominant graminoids can be extended by eCO_2, leading to the shading of late-season forbs, reduced gap availability and consequent reductions in diversity (Zavaleta et al., 2003). However, in other systems, eCO_2 has ameliorated N-induced losses in diversity (Reich, 2009), but in intact alpine grasslands, plant community composition changed and diversity declined only when eCO_2 and N were combined, but not when either was applied in isolation (Zhu et al., 2020).

Elevated CO_2 can also improve the efficiency of photosynthesis (Ainsworth & Long, 2005, 2021), increasing quantities of photosynthate C that may be invested by plants in P acquisition. Indeed, Stöcklin et al. (1998) demonstrated that in a P-limited calcareous grassland, belowground biomass increased by 36% under eCO_2, suggesting plants were utilising additional C resources to invest in root architecture for P acquisition (Stöcklin et al., 1998). As plant strategies for accessing P differ in their relative C requirements (Raven et al., 2018), species better able to capitalise on additional C resources may benefit at the expense of others (Polley et al., 2012), leading to shifts in community composition. Furthermore, responses of particular functional types to eCO_2 can be magnified when other nutrients are added, such as graminoids increasing with N (Grünzweig & Körner, 2003) or legumes increasing with P (Stöcklin et al., 1998). This suggests that eCO_2, in parallel with changes in nutrient availability, could lead to distinct plant communities that could not be predicted from responses to either eCO_2 or nutrients alone.

Overall, the potential roles of C supply in controlling rates of N and P acquisition (Cavagnaro et al., 2011; Jin et al., 2015) and the effects of N addition on P demand and uptake (Long et al., 2016; Menge & Field, 2007) suggest eCO_2 and its interactions with nutrient inputs

could substantively influence grassland community composition and diversity. However, to date, there is almost no understanding of how these two concurrent global change drivers impact community composition in P-limited grasslands.

Here, we explore the effects of eCO_2 and long-term N and P additions in two contrasting grasslands that are naturally P limited. The two grasslands, one acidic and the other limestone, share similar elevation, climate and grazing pressures. However, they have a number of key differences in plant communities and soil biogeochemistry that make them ideally suited for comparing the effects of eCO_2 and nutrient enrichment on two widely distributed grassland types. For instance, the limestone grassland is approximately twice as species rich as the acidic grassland and contains a broader range of plant functional types and, consequently, P-acquisition strategies. Furthermore, due in part to differences in pH and soil depth, P bioavailability differs greatly between the two grasslands (Table 1).

From these grasslands, we translocated plant–soil monoliths from an ongoing long-term (>25 years) nutrient manipulation field experiment into an outdoor Free Air CO_2 Enrichment (FACE) facility for 3 years. Plant communities were surveyed to determine how eCO_2, simulated N deposition, P addition and their interactions affected the community composition, biodiversity and functional composition of the two grasslands.

We hypothesised that:

1. Nutrient additions would significantly change plant communities and reduce biodiversity overall.
2. Elevated CO_2 would drive significant changes in grassland plant communities, with the more species-rich limestone grassland being more sensitive to eCO_2-induced changes.
3. There would be significant interactive effects of eCO_2 and nutrient additions on the community and functional composition of P-limited grasslands.

2 | METHODS

2.1 | Field site description

The acidic and limestone grasslands are co-located at the Wardlow Hay Cop long-term experiment located in the Peak District National Park, UK (Morecroft et al., 1994) and are naturally P limited (Carroll et al., 2003; Horswill, 2004; Keane et al., 2023). The limestone grassland plant community (National Vegetation Classification *Festuca-Avenula*, CG2d; Rodwell, 1992) is species rich (25 species m^{-2}) and grows on a shallow humic ranker soil, pH of ca. 6.8 (Horswill et al., 2008), that extends, on average, ~10 cm to the limestone bedrock beneath (Keane et al., 2020). The acidic grassland community (NVC *Agrostis-Galium*, U4e; (Rodwell, 1992)) is less rich in species (12 species m^{-2}) and contains more calcifuge species as it grows in a deep (>70 cm) cryptic podzol on silica-rich loess with a surface pH of ca. 4.4 (Horswill et al., 2008; Keane et al., 2020). The acidic soil is separated into an organic-rich A horizon (approx. 7–10 cm depth)

where the majority of biological activity occurs, and the deep mineral horizon isolates the plant roots from the limestone bedrock beneath (Keane et al., 2020). Note that the biodiversity in these grasslands occurs at a fine scale, so the m^{-2} richness values quoted are similar to those in the 0.35×0.35 m^2 soil-turf monoliths extracted and placed within the FACE facility (Figure 6).

2.2 | Long-term nutrient treatments

The nutrient addition experiment was established in 1995 using replicated 9-m^2 plots (Johnson et al., 1999). These receive one of four nutrient treatments, including a distilled water control (0N), a low N treatment (LN, 3.5 g m^{-2} year^{-1}) and a high N treatment (HN, 14 g m^{-2} year^{-1}) established to study the effects of atmospheric N deposition, and a P treatment (P, 3.5 g m^{-2} year^{-1}) to alleviate P limitation. Nutrients were applied as solutions at canopy height in the form of NH_4NO_3 and NaH_2PO_4. Nitrogen treatments have been applied monthly since 1995 and bimonthly since 2017. In the limestone grassland, due to a large biomass response to P addition in the first year, this treatment was ceased between 1996 and 2011 before being restarted. To date, N has been added to both grasslands for 25 years, P for 25 years for the acidic grassland and 11 years for the limestone grassland. As P is much less labile than N, the P-treated limestone plots have remained in a state of elevated P availability for the duration of the long-term experiment. Table 1 provides a summary of soil physico-chemical changes following long-term nutrient additions in both grasslands.

2.3 | Elevated CO_2 treatment

Full details of the MiniFACE system and the eCO_2 experiment are in Miglietta et al. (2001) and Keane et al. (2020), respectively. In brief, 10 replicate intact soil-turf monoliths (35 cm by 35 cm) were extracted from each nutrient treatment of both grasslands (80 monoliths in total) in 2017. Monoliths were taken to a depth of 10 cm in the limestone grassland to capture the full soil profile and to 20 cm in the acidic grassland to capture the organic horizon and the main rooting zone within the mineral horizon (Keane et al., 2020). These monoliths were transplanted into free-draining polypropylene mesocosm boxes. An additional 5 cm of limestone chippings was placed into the bottom of the limestone grassland mesocosms because, in the field, plant roots are regularly in contact with the bedrock. The same N and P treatments as in the Wardlow field experiment were applied monthly to the experimental mesocosms.

Mesocosms were housed outdoors at the Bradfield Environmental Laboratory research station, also in the Peak District (ca. 350 m asl), which shares similar climatic conditions and elevation as Wardlow (ca. 390 m asl). The mesocosm boxes were inserted into the soil, with the turf flush with surrounding vegetation to ensure normal thermal buffering (Keane et al., 2020). Mesocosms were

TABLE 1 Soil physico-chemical properties for both grasslands.

Soil property	Grassland	Nutrient treatment			
		0N	LN	HN	P
Total N (g kg^{-1})	Acidic	13.45 (±1.00)	11.94 (±0.59)	14.62 (±1.09)	11.71 (±0.58)
	Limestone	10.47 (±0.47)	12.04 (±0.66)	12.47 (±1.20)	10.93 (±0.74)
Total P (g kg^{-1})	Acidic	0.80 (±0.02)	0.94 (±0.03)	0.93 (±0.05)	1.16 (±0.17)
	Limestone	1.02 (±0.02)	0.81 (±0.07)	0.83 (±0.06)	1.19 (±0.05)
Extractable P (mg kg^{-1})	Acidic	30.37 (±3.95)	43.70 (±4.31)	53.49 (±9.43)	66.85 (±5.53)
	Limestone	6.85 (±0.25)	7.63 (±1.27)	8.64 (±1.60)	10.92 (±0.92)
Total extractable N (mg kg^{-1})	Acidic	16.05 (±6.49)	28.98 (±3.78)	87.38 (±18.41)	17.47 (±8.35)
	Limestone	11.31 (±3.75)	22.60 (±5.19)	62.75 (±6.99)	24.88 (±15.37)
pH	Acidic	4.57 (±0.05)	4.87 (±0.32)	4.07 (±0.08)	4.60 (±0.14)
	Limestone	7.04 (±0.05)	6.37 (±0.16)	6.04 (±0.26)	6.63 (±0.09)

Note: Data show mean values ± standard error of the mean. Soil total N and P were measured after 23 and 25 years of treatment, respectively; extractable P after 24 years; extractable N after 15 years; and soil pH after 26 years. While not all data was collected in the final year of the experiment, the plant communities have responded over many years to the treatments, and hence earlier long-term data remains valid for interpreting community responses. Extractable N from Lees (2013).

freely draining through holes in the base of the boxes that were covered in mesh voile to ensure roots did not grow out and come into contact with the FACE site soil. Mesocosms were paired based on productivity data and assigned to either ambient CO_2 (aCO_2, approximately 410 ppm) or elevated CO_2 (eCO_2, a target of 600 ppm). Elevated and ambient CO_2 treatments were separated by six metres of buffer space, with tall vegetation in between. Ambient CO_2 treatments were not downwind of the eCO_2 rings and were continuously monitored for CO_2 contamination, of which none was observed during the duration of the fumigation. CO_2 enrichment using the miniFACE system (Miglietta et al., 2001) started in April 2018 and continued for 3 years (2018, 2019 and 2020) for each growing season, with fumigation running between the start of April and the end of October during daylight hours.

2.4 | Data collection

The percentage cover of each plant species was determined from community composition surveys in July 2020, after 3 years of CO_2 fumigation and 25 years of nutrient manipulation (see Taylor et al., 2023 for dataset). Each mesocosm was split into a quadrat consisting of nine equally sized replicate squares, and the percentage cover was estimated for each species in each square. If estimated cover was over 5%, it was recorded to the nearest 5%, and if below 5%, data were recorded to the nearest single percent. The total cover of all species could exceed 100%. Bare soil and dead plant material were also recorded but not included in the analyses.

Each species was assigned to one of the following plant functional types (PFTs): forb, grass, sedge, legume, bryophyte, rush or unknown for later analyses. Due to time constraints, the cover of bryophytes was recorded, but species were not identified. As similar congeners, the grasses *Agrostis capillaris* and *Agrostis vinealis* were

not distinguished and instead aggregated as *Agrostis* spp. The rush functional type contains only one species, *Luzula campestris*. While *Helianthemum nummularium* and *Thymus praecox* both have woody tissue, they were included in the forb classification due to their functional similarities to other forbs. Unknown species were excluded from analyses, and the unknown PFT was included only to ensure that additional percentage cover was not incorrectly attributed to another PFT. The percentage of the plant community that was unknown was very low and accounted for 0.1% of total mesocosm cover on average for both grasslands, with a maximum of 1.8% and 2.3% for any one mesocosm of the limestone and acidic grasslands, respectively.

2.5 | Statistical analyses

2.5.1 | Community composition

All analyses were performed in R version 3.6.3 (R Core Team, 2021) in the 'tidyverse' environment (Wickham et al., 2019). Differences in community composition were determined by analysing species abundance data using non-metric multidimensional scaling (NMDS), whereby the larger the difference in NMDS axis values, the more dissimilar the plant communities (Anderson, 1971). A Bray–Curtis dissimilarity index was used, as this is deemed appropriate for ecological data where multiple scarce species may be present (Anderson, 1971; Klupar et al., 2021).

The Bray–Curtis dissimilarity values were min-max normalised prior to NMDS analysis to ensure that each variable contributed equally towards the NMDS. NMDS analyses were performed using the 'metaMDS' function from the 'vegan' package in R (Dixon, 2003). The NMDS reduced the data dimensionality into two dimensions by using 100 model iterations and, in doing so, generated stress values below the generally accepted 0.2 threshold (Klupar et al., 2021) for both grasslands.

To quantify the effects of nutrients, CO_2 treatment and their interactions on plant communities, a permutational multivariate analysis of variance (PERMANOVA) test was used. PERMANOVA is a non-parametric test that partitions variation across a multivariate data cloud and can use a Bray–Curtis dissimilarity matrix (Anderson, 2017); hence it is complementary to an NMDS analysis. PERMANOVAs test the null hypothesis that the centroid of each treatment's ordination in the dissimilarity matrix is not different (Anderson, 2017); hence, a significant result from a PERMANOVA suggests that treatment groups are significantly dissimilar. The 'adonis2' function from the 'vegan' package was used to analyse the Bray–Curtis dissimilarity matrix of each mesocosm's plant community, calculated from 999 permutations.

2.5.2 | Species associations with treatments

Principal component analyses (PCA) were used to visualise the relative association of each species to the community of a particular treatment combination. The PCA analyses presented here act only as a visualisation of the data; hence, statistical analyses on the PCA output were not performed. PCAs were conducted using the 'PCA' function in the 'FactoMineR' package in R (Lê et al., 2008), and as with the NMDS, the dissimilarity matrices were min–max normalised prior to analysis.

2.5.3 | Plant functional composition

The mean cover of each PFT in a mesocosm was calculated, and zero-loaded PFTs were excluded from analyses to improve the fit of the full statistical models; hence, in the acidic grassland, legumes and sedges were not analysed, nor were rushes in the limestone grassland. Limestone grassland data were natural log +1 transformed, but untransformed acidic grassland data was used as the latter dataset more closely resembled that drawn from a normal distribution once zero-loaded PFTs were removed.

Linear mixed effect models (LMEs) were fitted to the data with summed PFT cover as the response variable and PFT, nutrient and CO_2 treatment as categorical fixed effects along with their factorial interaction terms, and pair ID as a random effect term. The models were fit using the 'lmer' function from the 'lme4' package in R (Bates et al., 2015). The data were split into different functional types for the purposes of multiple comparisons, and the 'emmeans' package (Lenth et al., 2021) was used to determine the significance of pairwise differences with the Tukey multiple comparison of means test.

2.5.4 | Grassland diversity

Species richness was calculated by summing the number of species present in each mesocosm. To assess how species richness across grasslands was affected by eCO_2, nutrient additions and their interactions, a generalised linear mixed effects model with a Poisson distribution was fitted to the data using the 'glmer' function from the 'lme4' package (Bates et al., 2015). The suitability of the fitted model to the data was visually assessed and was deemed appropriate without data transformation.

The Simpson's diversity index is a measure of community diversity that considers species richness and their relative abundance, thus providing a means of assessing community evenness and dominance (Simpson, 1949). The Simpson's diversity index is less sensitive to the occurrence of multiple rare species than the Shannon index (Shannon, 1948; Spellerberg & Fedor, 2003); hence, we deemed it more appropriate for our grassland communities, which are comparatively rich and contain many species of low abundance. The Simpson's index value (D) quantifies the probability of two independently sampled individuals being the same species; hence, D decreases with diversity. We therefore use 1-D to make interpretation more intuitive (i.e. higher values represent greater diversity). This was calculated using the 'diversity' function from the 'vegan' package in R. As 1-D represents proportional data, we logit-transformed the data prior to its inclusion in a linear mixed effects model with the same structure as for PFT composition.

3 | RESULTS

3.1 | Community composition

In the limestone grassland, nutrient treatments created plant communities significantly dissimilar from one another (PERMANOVA, $df = 3$, 28, $F = 8.4$, $p < .01$), and eCO_2 treatment also produced communities that were significantly dissimilar to aCO_2 treatments (PERMANOVA, $df = 1$, 28, $F = 2.3$, $p < .05$) (Figure 1a). The different nutrients produced distinct communities compared to the 0N control, with divergent trajectories in the ordination space, highlighting that N- and P-treated communities also differed from each other. There was no significant interaction between CO_2 and nutrient treatments at the community level (PERMANOVA, $df = 3$, 28, $F = 0.4$, $p > .05$), though eCO_2 appeared to have a stronger effect in the LN and HN treatments compared to the 0N and P treatments, with eCO_2-treated communities generally having more positive score positions on the NMDS1 axis (Figure 1a).

Likewise, in the acidic grassland, nutrient additions led to significantly dissimilar communities (PERMANOVA, $df = 3$, 28, $F = 5.0$, $p < .01$), though ordinations between nutrient treatments overlapped considerably and were less distinct than in the limestone grassland (Figure 1b). Elevated CO_2-treated communities were significantly different from their aCO_2 counterparts (PERMANOVA, $df = 1$, 28, $F = 2.4$, $p < .05$), notably in the LN treatments and, to a lesser extent, the P treatments. There were no significant interactions between CO_2 and nutrients on community composition (PERMANOVA, $df = 3$, 28, $F = 1.5$, $p > .05$) (Figure 1).

(a) Limestone community composition

(b) Acidic community composition
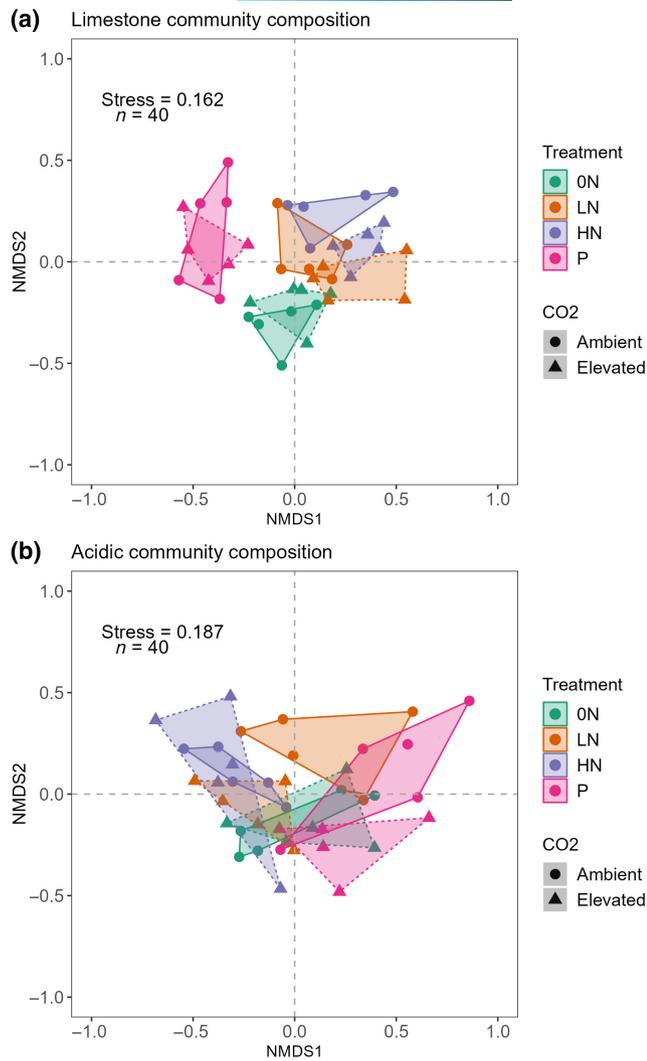
FIGURE 1 Non-metric multidimensional scaling (NMDS) ordinations of community composition for the (a) limestone and (b) acidic grasslands, where each data point represents the community of an individual mesocosm. The NMDS plot visualises the dissimilarity between plant communities under different nutrient (0N, LN, HN and P) and CO_2 (ambient and elevated) treatment combinations. Each treatment combination occupies discrete space in the ordination plot, highlighted by the shaded polygons, and the further apart these are from other polygons, the more dissimilar the plant communities.

3.2 | Species associations

In the limestone grassland, the PCA shows that individual species can have strong associations with particular CO_2 and nutrient treatment combinations. Fewer species are associated with N addition, with many instead associating with 0N or P treatment (Figure 2), reflecting the less diverse N-treated plant communities (see also Figure 6). The 0N treatments were characterised by many typical limestone grassland forbs, such as *Linum catharticum*, *Cerastium fontanum* and *Euphrasia officinalis* (Figure 2; Figure S3). Almost all limestone grass species were positively associated with P addition, including *Koeleria*

macrantha, *Agrostis* spp., *Briza media* and *Anthoxanthum odoratum* (Figure 2; Figure S3).

Legumes, including *Lotus corniculatus* and *Anthyllis vulneraria*, are generally associated more positively with eCO_2 than aCO_2 treatments, and *Trifolium repens* and *T. pratense* are strongly associated with P addition, especially when combined with CO_2 enrichment (Figure 2; Figure S3). Furthermore, combined eCO_2 and N treatments (LN and HN) were associated with sedge species, notably *Carex flacca* and *Carex panicea* (Figure 2), and at the functional level, sedges and grasses showed divergent associations, with arrows ordinated in opposite directions (Figure S3).

In the acidic grassland, only the stress-tolerant grasses *Nardus stricta* and *Festuca ovina* and the sedge *Carex pilulifera* were strongly associated with the HN treatments (Figure 3). Species from a range of functional types were associated with P addition, including legumes (*Trifolium repens*), grasses (*Poa* spp.) and forbs (*Plantago lanceolata*, *Rumex acetosa*). As the effects of nutrient treatments on community composition were less distinct in the acidic than the limestone grassland (Figure 1), similarly, the associations of species and functional types with particular treatments were also less apparent in the acidic compared to the limestone grassland (Figure 3; Figure S6).

Many species are associated most strongly with aCO_2-0N and aCO_2-LN, or with P treatment at either CO_2 concentration. These species represent a mix of functional types, including grasses such as *Deschampsia flexuosa* and *Anthoxanthum odoratum*, bryophytes and some forbs such as *Campanula rotundifola* (Figure 3). In contrast, few species associated with eCO_2 combined with any of the 0N, LN and HN treatments (Figure 3), which clustered in a more similar and compact ordination space than their aCO_2 counterparts, suggesting eCO_2 may modify a species' association with nutrient treatments (Figure 3). In particular, the two LN treatments are oriented in opposite directions depending on CO_2 treatment, with more species associating with aCO_2 than eCO_2 (Figure 3), potentially reflecting the differences in community composition (Figure 1).

3.3 | Plant functional type composition

There were significant effects of nutrient treatments alone (LME, $df = 3$, 160; $F = 2.9$; $p < .05$) but not of eCO_2 in the absence of nutrient treatments (LME, $df = 1$, 160; $F = 2.0$; $p > .05$) (Figure 4). Elevated CO_2 and nutrient treatments also interacted to significantly alter the functional composition of the limestone grassland (LME, $df = 12$, 160; $F = 1.9$; $p < .05$) (Figure 4)—see PFT-specific details below.

Limestone grasses were significantly less abundant with eCO_2 compared to aCO_2 (LME $df = 1$, 28; $F = 4.9$; $p < .05$), with the CO_2 effect amplified as N loading increased (Figure 4; Figure S1). Under aCO_2, limestone grass cover significantly increased with nutrient additions (LME, $df = 3$, 28; $F = 7.5$; $p < .001$) from 45.3% in 0N to 47.6%
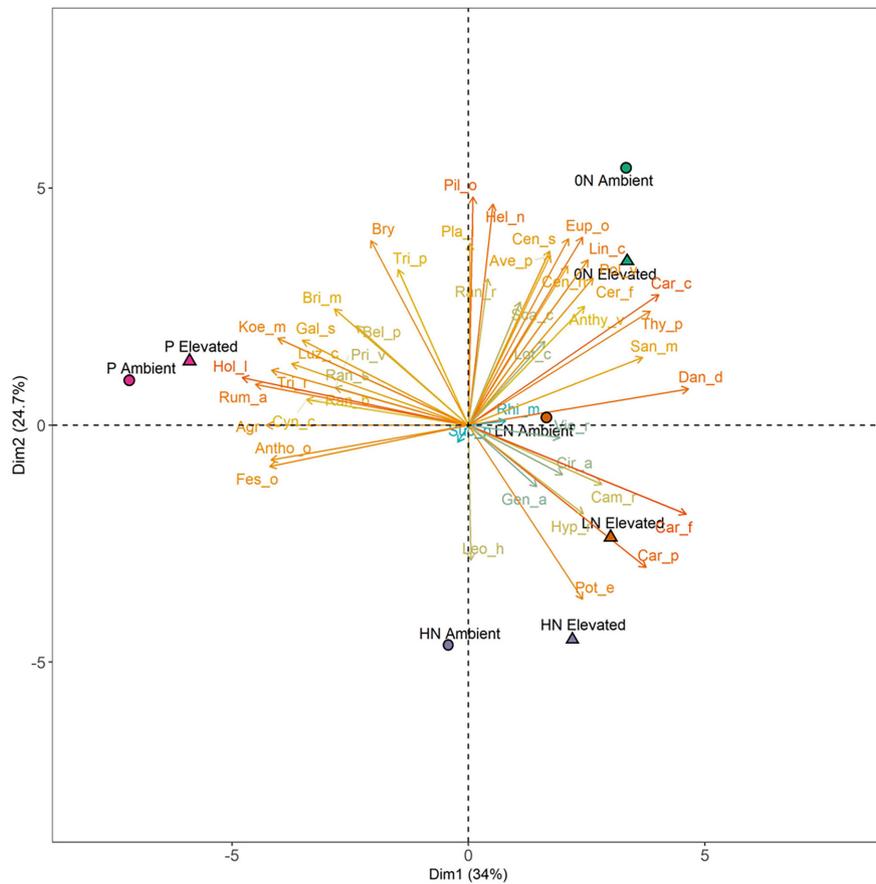
FIGURE 2 A principal component analysis visualising the association of limestone grassland species with the mean community composition of nutrient-CO_2 treatment combinations. Each recorded species has an arrow orientated towards the treatment that the species most strongly associates with, which can be interpreted as a species' 'preferred' treatment combination. The length of the arrow and the warmth of its colour (blue to red) are indicative of the strength of association, such that longer and redder arrows are more strongly associated with a treatment than shorter, bluer arrows. Agr, *Agrostis spp*; Antho_o, *Anthoxanthum odoratum*; Anthy_v, *Anthyllis vulneraria*; Ave_p, *Avenula pratense*; Bel_p, *Bellis perennis*; Bri_m, *Briza media*; Bry, Bryophytes; Cam_r, *Campanula rotundifolia*; Car_c, *Carex caryophyllea*; Car_f, *Carex flacca*; Car_p, *Carex panicea*; Cen_n, *Centaurea nigra*; Cen_s, *Centaurea scabiosa*; Cer_f, *Cerastium fontanum*; Cir_a, *Cirsium acuale*; Cyn_c, *Cynosurus cristatus*; Dan_d, *Danthonia decumbens*; Eup_o, *Euphrasia officinalis*; Fes_o, *Festuca ovina*; Gal_s, *Galium sterneri*; Gen_a, *Gentianella amarella*; Hel_n, *Helianthemum nummularium*; Hol_l, *Holcus lanatus*; Hyp_r, *Hypochaeris radicata*; Koe_m, *Koeleria macrantha*; Leo_h, *Leontodon hispidus*; Lin_c, *Linum catharticum*; Lot_c, *Lotus corniculatus*; Luz_c, *Luzula campestris*; Pil_o, *Pilosella officinarum*; Pla_l, *Plantago lanceolata*; Pol_v, *Polygala vulgaris*; Pot_e, *Potentilla erecta*; Pri_v, *Primula veris*; Ran_b, *Ranunculus bulbosus*; Ran_r, *Ranunculus repens*; Ran_s, *Ranunculus sardous*; Rhi_m, *Rhinanthus minor*; Rum_a, *Rumex acetosa*; San_m, *Sanguisorba minor*; Sca_c, *Scabiosa columbaria*; Suc_p, *Succisa pratensis*; Thy_p, *Thymus praecox*; Tri_p, *Trifolium pratense*; Tri_r, *Trifolium repens*; Vio_r, *Viola riviniana*.

in LN and 56.2% in HN and was highest at 67.7% in the P treatment (Tukey, $p < .05$).

In contrast to grasses, limestone sedge cover increased significantly from 27.9% under aCO_2 to 37.4% under eCO_2, averaged across all nutrient treatments (LME, $df = 1$, 28; $F = 13.5$; $p < .01$) (Figure 4; Figure S1). These increases tended to be greater with N additions, though this interaction was not statistically significant (LME, $df = 3$, 28; $F = 0.4$; $p > .05$) (Figure 4; Figure S1). Sedge cover was also significantly affected by nutrient treatments (LME, $df = 3$, 28; $F = 73.5$; $p < .001$), generally increasing with N addition (0N 31.7% < LN 42.2% < HN 48.8%), but in marked contrast to the grasses (Figure 4; Figure S1), was significantly lower in P (7.9%) treatments (Tukey, $p < .05$).

Overall, in the limestone grassland, the contrasting responses of grasses and sedges to eCO_2 and the amplification of this by N

meant that grass dominance declined with eCO_2 and increased N loading, while sedges increased. This occurred to the extent that in the eCO_2-HN treatment, sedges overtook grasses as the most abundant PFT (Figure 4; Figure S1).

In contrast, forb cover was unaffected by CO_2 treatment (LME, $df = 1$, 28; $F = 1.4$; $p > .05$) or its interactions with nutrient additions (LME, $df = 3$, 28; $F = 0.1$; $p > .05$) (Figure 4), but did significantly decline with nitrogen additions (LME, $df = 3$, 28; $F = 9.4$; $p < .001$), with the greatest difference between 0N (65.7%) and HN treatments (36.5%) (Tukey, $p < .05$).

Limestone legume cover did not change significantly with nutrient treatments (LME, $df = 3$, 32; $F = 2.7$; $p > .05$) or CO_2 treatment (LME, $df = 1$, 32; $F = 4.1$; $p > .05$) alone, though individual species showed contrasting responses to eCO_2. For example,
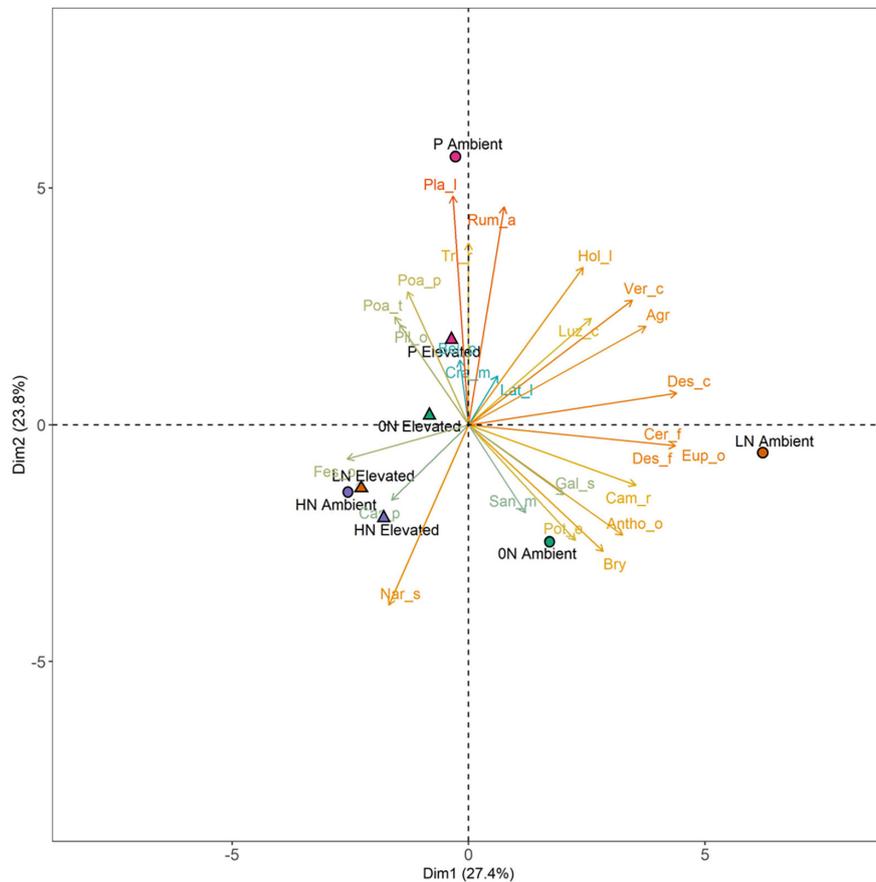
FIGURE 3 A principal component analysis visualising the association of acidic grassland species with the mean community composition of nutrient-CO_2 treatment combinations. Each recorded species has an arrow orientated towards the treatment that the species most strongly associates with, which can be interpreted as a species' 'preferred' treatment combination. The length of the arrow and the warmth of its colour (blue to red) are indicative of the strength of association, such that longer and redder arrows are more strongly associated with a treatment than shorter, bluer arrows. Agr, *Agrostis spp*; Antho_o, *Anthoxanthum odoratum*; Bel_p, *Bellis perennis*; Bry, Bryophyte; Cam_r, *Campanula rotundifola*; Car_p, *Carex pilulifera*; Cer_f, *Cerastium fontanum*; Cra_m, *Crataegus monogyna*; Des_c, *Deschampsia cespitosa*; Des_f, *Deschampsia flexuosa*; Eup_o, *Euphrasia officinalis*; Fes_o, *Festuca ovina*; Gal_s, *Galium saxatile*; Hol_l, *Holcus lanatus*; Lat_l, *Lathyrus linifolius*; Luz_c, *Luzula campestris*; Nar_s, *Nardus stricta*; Pil_o, *Pilosella officinarum*; Pla_l, *Plantago lanceolata*; Poa_p, *Poa pratensis*; Poa_t, *Poa trivialis*; Pot_e, *Potentilla erecta*; Rum_a, *Rumex acetosa*; San_m, *Sanguisorba minor*; Tri_r, *Trifolium repens*; Ver_c, *Veronica chamaedrys*.

Lotus corniculatus and *Anthyllis vulneraria* increased by 2.1% and 1.6%, respectively, whereas *Trifolium pratense* declined by 3.2% (Figure S4). Elevated CO_2 significantly interacted with nutrient treatments, with substantial increases (more than tripling) in legume cover in the combined eCO_2 and P treatment (LME, $df = 3$, 32; $F = 3.3$; $p < .05$) from 11.1% in P-aCO_2 to 36.9% in the P-eCO_2 treatment (Tukey, $p < .05$).

Bryophyte cover was significantly affected by CO_2 (LME, $df = 1$, 32; $F = 5.4$; $p < .05$) and nutrient treatments (LME, $df = 3$, 32; $F = 8.3$; $p < .001$) but not their interaction (LME, $df = 3$, 32; $F = 0.1$; $p > .05$), generally decreasing in response to both eCO_2 and N additions singly (Figure 4).

Functional composition in the acidic grassland was also significantly affected by nutrient treatment (LME, $df = 9$, 124; $F = 5.9$; $p < .001$), though in contrast to the limestone grassland, there were no significant effects of eCO_2 treatment alone on any PFT (LME, $df = 1$, 124; $F = 1.2$; $p > .05$), nor did CO_2 interact with nutrient

treatments to affect PFT composition (LME, $df = 3$, 124; $F = 0.6$; $p > .05$) (Figure 5).

Acidic grass cover was significantly affected by nutrient treatment (LME, $df = 3$, 28; $F = 7.3$, $p < .001$), being highest in HN (94.8%), which was significantly more than the LN treatment (77.6%), but not 0N (80.3%) or P treatments (86.9%) (Tukey, $p < .05$). Likewise, forb cover was significantly affected by nutrients (LME, $df = 3$, 32; $F = 5.0$; $p < .01$) declining with N addition (0N 28.4% > LN 27.03% > HN 8.6%) and also with P (24.1%). Bryophytes were not significantly affected by nutrients (LME, $df = 3$, 28; $F = 1.0$; $p > .05$). Rush (*Luzula campestris*) abundance was significantly affected by nutrients (LME, $df = 3$, 28; $F = 12.5$; $p < .001$), where N addition decreased cover, significantly so with HN, and P addition increased it (Tukey, $p < .05$) (Figure 5).

While there were significant changes in community composition in response to eCO_2 (Figure 1b), these were either of too small a magnitude or occurred among species sharing a functional type,

Limestone PFT composition
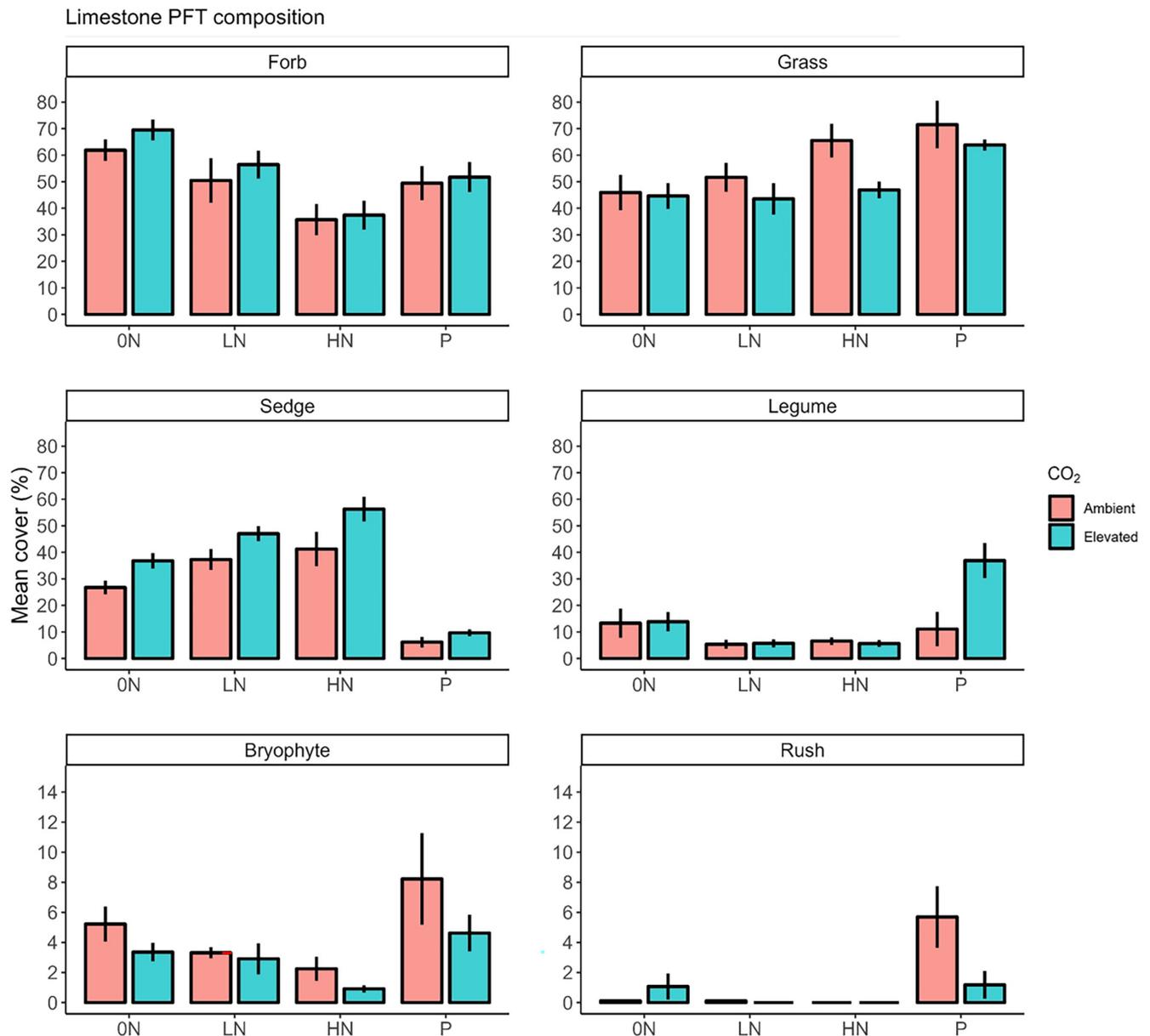
FIGURE 4 Plant functional composition in the limestone grassland and how the mean cover of each functional type is affected by elevated CO_2 and nutrient treatments (0N, LN, HN and P). Error bars represent the standard errors of the means ($n=5$). Note that the bryophyte and rush functional types have a different y-axis scale than the more abundant plant functional types.

such that they did not translate to significant shifts in PFT (Figure 5). For instance, the largest within-PFT divergence with eCO$_2$ was with the grasses *Festuca ovina* (an increase of 8.9%) and *Anthoxanthum odoratum* (a decrease of 7.9%) (Figure S7).

A qualitative summary of the main effects of nutrient and CO_2 treatment on the cover of each grassland's PFT is provided in Table S1.

3.4 | Grassland biodiversity

Across both grasslands, mean species richness was significantly affected by nutrient treatments (GLM, $df=3$; $\chi^2=10.3$; $p<.05$), being lower in the HN than all other treatments (GLM, $Z=2.2$; $p<.05$)

(Figure 6). HN-treated communities had on average five fewer (−21.7%) species per mesocosm than their 0N counterparts in the limestone grassland and three fewer (−25.0%) in the acidic grassland (Figure 6). However, eCO$_2$ had no significant effect on richness (GLM, $df=1$; $\chi^2=0.1$; $p>.05$), nor did the interactions between CO_2 and nutrient treatments (GLM, $df=3$; $\chi^2=0.7$; $p>.05$).

The effects of nutrient treatments and eCO$_2$ on Simpson's diversity closely resembled the species richness results. There were significant effects of nutrient treatment on limestone (LME, $df=3$, 28, $F=8.3$, $p<.001$) and acidic grassland (LME, $df=3$, 32, $F=8.4$, $p<.001$) diversity. In the limestone grassland, HN- and P-treated communities had significantly lower Simpson's diversity than 0N (HN; $t(28)=1.6$, $p<.01$, P; $t(28)=4.1$, $p<.001$), and in the acidic
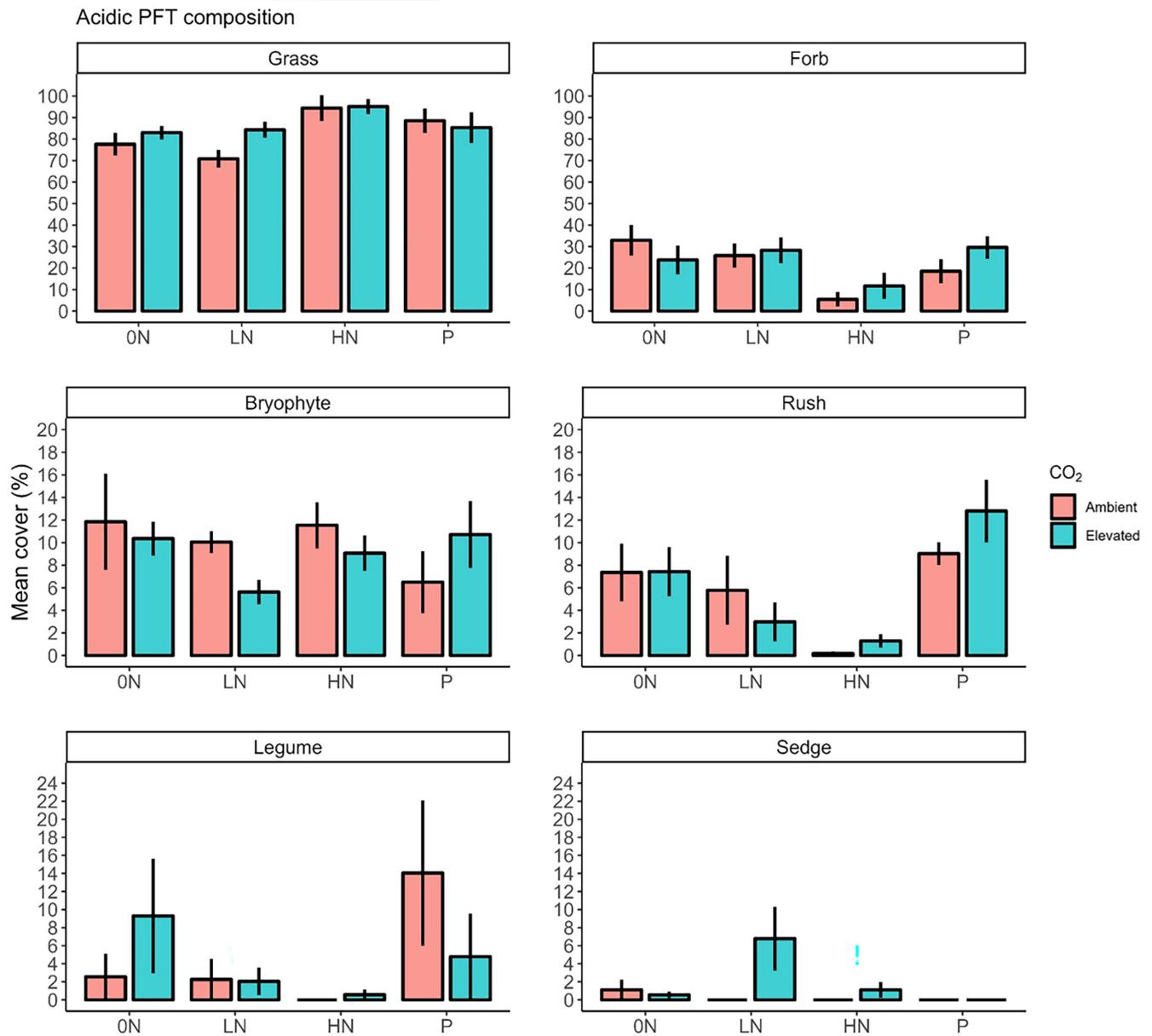
Acidic PFT composition

FIGURE 5 Plant functional composition in the acidic grassland and how the mean cover of each functional type is affected by elevated CO_2 and nutrient treatments (0N, LN, HN and P). Error bars represent the standard errors of the means ($n=5$). Note that the y-axis scale differs across functional types.

grassland, HN treatment significantly reduced Simpson's diversity compared to all other nutrient treatments ($t(32)=2.8$, $p<.01$) (Figure 6). However, there were no significant responses to eCO_2 in the limestone (LME, $df=1$, 28, $F=2.1$, $p>.05$) or acidic grassland (LME, $df=1$, 32, $F=0.0$, $p>.05$), and neither did eCO_2 interact with nutrient treatments in either grassland (acidic LME; $df=3$, 32, $F=0.3$, $p>.05$, limestone LME; $df=3$, 28, $F=1.0$, $p>.05$) (Figure 6).

4 | DISCUSSION

Our study shows that eCO_2 after only 3 years can cause substantial shifts in the community composition of two contrasting

grasslands and can modify the responses of different plant functional types to N and P additions ($CO_2 \times$ nutrient interactions), resulting in alterations to functional composition. After 25 years of N enrichment and (to a lesser extent) P addition, both grassland communities became less diverse and more graminoid dominated as forbs declined. Both direct and interactive responses to eCO_2 were generally stronger in the limestone grassland, where eCO_2 combined with N enrichment to facilitate a major shift in PFT dominance, with a substantial replacement of grasses with sedges, leading to a sedge-dominated grassland. Elevated CO_2 also combined with P enrichment to dramatically promote legume abundance in the limestone grassland.

FIGURE 6 Species richness and Simpson's diversity for the acidic and limestone grasslands across nutrient and CO_2 treatments. The smaller, semi-transparent data points show the richness and diversity, respectively, of individual mesocosms. The larger points represent the mean richness and diversity across all mesocosms sharing the same treatment and are presented with their respective standard errors ($n = 5$).
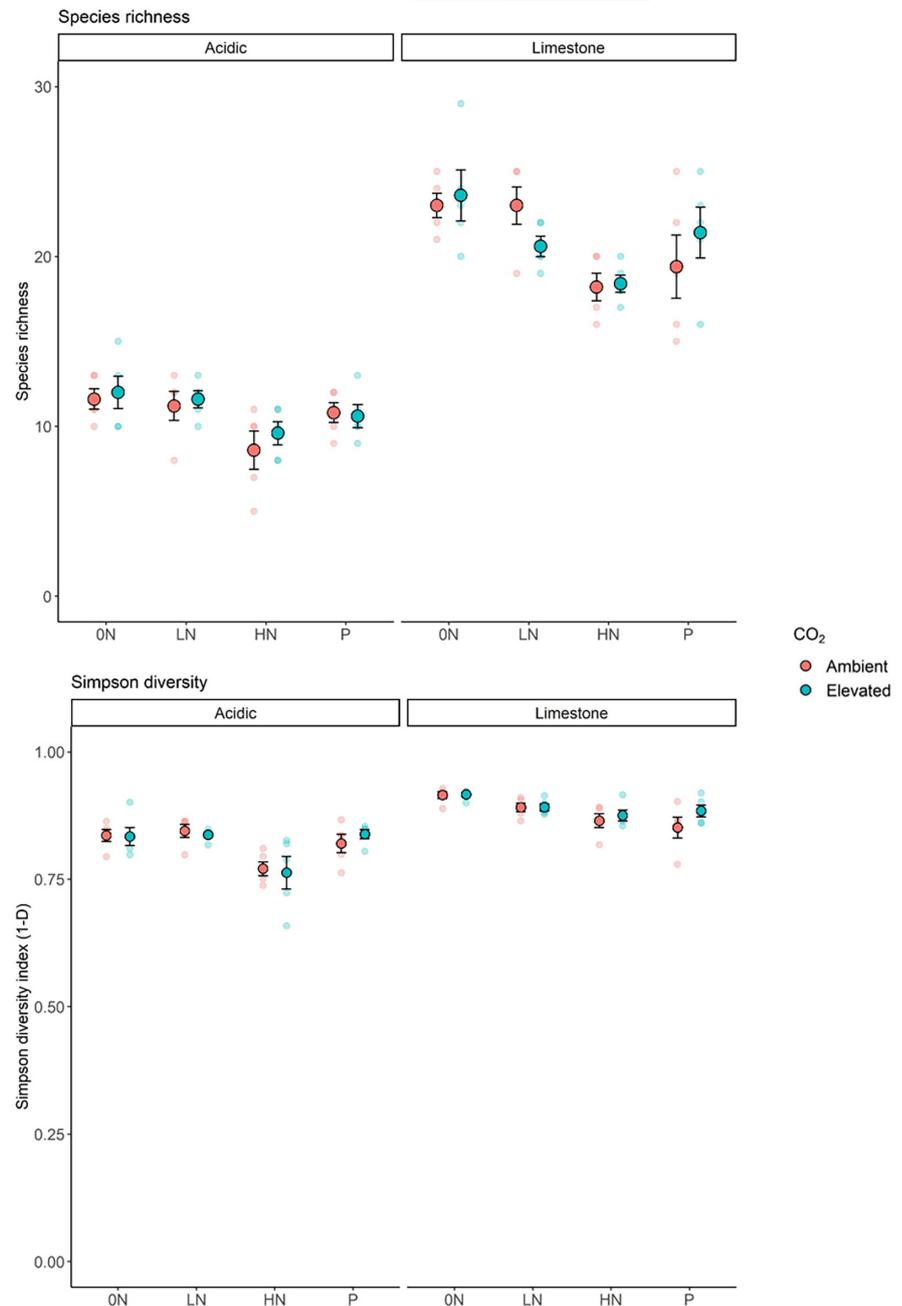
4.1 | Grassland community responses to long-term nutrient additions

In agreement with our first hypothesis, both grasslands saw significant reductions in richness and diversity with nutrient additions, particularly of N, which were attributable to substantial declines in forbs, leading to graminoid-dominated communities. Similar responses have been previously observed (Lu et al., 2021; Stevens et al., 2004; You et al., 2017), including at the Wardlow acidic and limestone grassland sites (Carroll et al., 2003; Lee & Caporn, 1998), from which our mesocosms originate. Accordingly, N and P additions led to consistent and significant changes in the community and functional composition of both grasslands.

These effects were clearer in the limestone grassland, where N addition had a larger impact on species richness, potentially due to greater sensitivity to changes in pH. Limestone grassland communities can contain many calcicolous species, making them susceptible to even small decreases in pH resulting from N-induced acidification (Tian et al., 2021), such as with LN and HN additions in our experiment (Table 1). Forb reductions may arise from acidification-induced depletions in base cation availability observed at the site (Horswill et al., 2008), creating conditions suitable for stress-tolerant graminoids (Stevens et al., 2018). Furthermore, N-induced acidification of calcareous soils can increase macronutrient availability, such that conditions for N and P uptake improve and can be exploited to differing

extents by species capable of dominating the plant community (Bobbink, 1991; Stevens et al., 2011).

Such mechanisms may apply to the acidic grassland, where N additions favoured grasses such as *Nardus stricta* and *Festuca ovina*. In the limestone grassland, however, sedges such as *Carex panicea* and *Carex flacca* increased, which may be able to withstand N-induced acidification (Zhang et al., 2023) and more effectively scavenge the limiting P resource as N enrichment increases P demand (Horswill et al., 2008; Phoenix et al., 2004) through stimulation of dauciform root production that releases exudate bursts of organic acids (Ballard, 2001; Johnson, 1998; Shane et al., 2006). Conversely, we found N addition reduced legume abundance in both grasslands, potentially as the benefits of rhizobial symbioses become functionally redundant (Regus et al., 2017).

Provision of the most limiting nutrient to a grassland can lead to increased dominance of the fastest-growing species. For instance, Bobbink (1991) demonstrated how N additions to an N-limited grassland allowed *Brachypodium pinnatum* to obtain large amounts of N, grow fast and dominate and reduce diversity. Similarly, we found that P additions to our P-limited ecosystems led to grass dominance and reduced diversity, particularly in the limestone grassland, where *Anthoxanthum odoratum*, *Poa* and *Agrostis* species were likely better able to capitalise on the increased P supply and outcompete other species (Bobbink, 1991; Bobbink et al., 2010; Lu et al., 2021; You et al., 2017). Stiles et al. (2017) report similar reductions in diversity of up to 21% resulting from P enrichment in a P-limited upland grassland, emphasising the essential role of P limitation in maintaining the diversity of these ecosystems (Ceulemans et al., 2013, 2014; Stiles et al., 2017).

Given that our communities were surveyed after 25 years of nutrient enrichment, declines in richness may also be a result of longer-term effects on reproduction, such as depleted seedbanks as protective seed coatings become more decomposable under high N conditions and flower numbers of forbs potentially being reduced (Basto et al., 2015). These indirect effects on plant fitness could mean that the reductions in grassland biodiversity we report here are unlikely to be quickly reversed should nutrient loading decline.

4.2 | Responses of grassland communities to elevated CO_2

Elevated CO_2 led to significant changes in the community composition of both grasslands, and in support of our second hypothesis, limestone communities did appear more sensitive to eCO_2 treatment overall, where there was more consistent divergence of ambient and elevated CO_2 communities across treatments and greater changes to functional composition (Figures 1 and 4). Such responses were, however, not accompanied by eCO_2-driven reductions in richness or diversity in either grassland (Figure 6).

The most notable functional consequence of eCO_2 was a significant reduction in grass, combined with a simultaneous increase in sedge cover in the limestone grassland. In particular, and as

highlighted by the PCA visualisation (Figure 2), *Carex panicea* and *C. flacca* are strongly associated with eCO_2 treatments. This result is in broad agreement with Stöcklin et al. (1998), who also observed a very strong response of *C. flacca* to eCO_2 in a limestone grassland community. Sedges are well adapted to low-P conditions (Güsewell, 2017; Shane et al., 2006; also evidenced by their comparative absence in P treatments) and form dauciform root structures that release large quantities of carbon-based exudates in pulses (Shane et al., 2006). It may be that sedges are using the additional C supply from eCO_2 to acquire more of the limiting P nutrient via this mechanism, increasing their competitive ability against soil microbes and other plant species and hence increasing in abundance with eCO_2.

Indeed, previous research on the same limestone grasslands as this study has shown that the three most dominant *Carex* species, *C. flacca*, *C. panicea* and *C. caryophyllea*, all form dauciform root structures in response to P limitation (Ballard, 2001; Johnson, 1998), and they proliferate locally where P limitation is most intense (Johnson, 2007). Furthermore, in a pot experiment with soil and plants, again from the same limestone grassland, Ballard (2001) showed that eCO_2 also increased dauciform root production and activity, significantly so with our most CO_2-responsive species, *Carex flacca*. This was associated with increased shoot P content and hence indicative of greater mobilisation of soil P stores. Dauciform root surface enzyme activity also benefitted from eCO_2, with concurrent increases in phosphomonoesterase and phosphodiesterase activity (both are P-mobilising enzymes) in *C. panicea* and *C. caryophyllea* (Ballard, 2001).

This is further supported by our own recent work showing that overall investment in soil P-cycling enzymes (from both plant and microbial origins) significantly increases under eCO_2—an effect magnified by N additions, which doubled acid phosphatase activity (Keane et al., 2020). The previously observed increases in dauciform root production would likely increase sedge competition for inorganic and organic P sources. The pulses of carbon-rich exudation may increase the desorption of P from mineral forms (Lambers et al., 2008), while the greater phosphatase production may promote greater access to organic P (Ballard, 2001). We suggest that the presence of dauciform roots may be key to allowing *Carex* species to increase in abundance under elevated CO_2 as they overwhelm competition with soil microbes for P, consistent with our recent study suggesting that above-ground biomass responses to eCO_2 in both grasslands are mediated by strong microbial competition for P (Keane et al., 2023). The evidence that these low-P specialists are particularly responsive to eCO_2 may have important implications for plant communities in biodiverse P-limited ecosystems more generally. Where such specialist P-mining species are a major component of plant communities, eCO_2 may provide a means of alleviating P limitation and promoting ecosystem productivity (Reichert et al., 2022).

Beyond this substantial shift in the abundance of grasses versus sedges, eCO_2 led to contrasting within-PFT responses. This meant that for eCO_2, there were few significant changes at the PFT level, despite sometimes large species-level changes. Such responses were

especially prevalent in the broadest functional group, the forbs, for which approximately equal numbers of species responded positively to eCO_2 as they did negatively, leading to no significant changes at the functional level in either grassland (Figures S4 and S7). These within-PFT shifts suggest changes in competition between species of the same PFT, though given that P-acquisition adaptations tend to be more similar within PFTs (Phoenix et al., 2020), it is harder to explain these shifts based on the acquisition of the limiting P. It is important to note that apparently negligible responses of a PFT to eCO_2 does not mean that there are no changes in the abundance of species within that PFT (Hanley et al., 2004).

4.3 | Elevated CO_2 and nutrient interactions

The NMDS, PCA and functional analyses all suggest a clear interactive effect of eCO_2 and N additions on the cover of limestone grasses and sedges. In all nutrient treatments, eCO_2 increased sedge and decreased grass abundance, but the magnitude of these changes was amplified by the two N treatments, increasingly so by the HN compared to the LN (Figure 4; Figure S1). Ultimately, combined eCO_2 and HN treatments resulted in a grassland dominated by sedges, not grasses. While elevated CO_2 may increase the amount of photosynthate C available for P liberation and uptake mechanisms, simultaneous N loading could dictate the resulting outcome of competition for P. As N additions can elevate P demand (Long et al., 2016), eCO_2 effects on P uptake may become more important, meaning that plants could respond more strongly to eCO_2 if also combined with elevated N availability. This may be especially prevalent where plant P acquisition depends on the cooperation of a mutualist that may be adversely impacted by N availability.

In grasses, P uptake by arbuscular mycorrhizal fungi (AMF) may decline with N addition, as soil acidification, such as at Wardlow (Table 1), can inhibit the colonisation of roots by mycorrhiza and restrict growth of fungal biomass (Pan et al., 2020), and fungal communities shift towards slower P-cycling potentials as pathogenic fungi replace mutualists (Lekberg et al., 2021). In contrast, unlike most grassland plants, sedges seldom form mycorrhizal symbioses, with P uptake instead mediated by direct plant control of organic acid and phosphatase enzyme secretions (Ballard, 2001; Johnson, 1998; Shane et al., 2006). Therefore, the interactions of eCO_2 and N additions may disproportionately benefit sedges at the expense of grasses, as direct P mining is not dependent on the cooperation of fungal partners (Lambers et al., 2008) and is more capable of outcompeting soil microbes for P. Further research is needed to examine potential changes in the relative effectiveness of fungal P uptake versus direct plant P uptake under combined eCO_2 and N additions.

Finally, the most striking CO_2–nutrient interaction occurred when eCO_2 was combined with P treatments in the limestone grassland (Figure 4). In the eCO_2-P treatments, legumes, which form a minor component of the community (5.6%–13.8%), significantly increased to ~40% cover—over triple the cover of their counterpart in the aCO_2-P treatments (11.1%) (Figure 4). Others have suggested that legume growth in calcareous grasslands could be co-limited by C and P availability (Niklaus et al., 1998), and our data support that hypothesis because legume abundance is not increased by eCO_2 or P alone, but is substantially increased by their combination. Nitrogen fixation is an energetically costly process with respect to both C (Minchin & Witty, 2005) and P resources (Liu et al., 2018); hence, the simultaneous provision of C and P may represent improved conditions for legume proliferation. Because legume cover can have substantial impacts on N availability and cycling in grassland systems (Cowling, 1982), such a large increase in their abundance is likely to stimulate productivity should N become the limiting factor for growth. This may be particularly important under eCO_2, where carbon dilution of plant tissue may lead to progressive N limitation (Luo et al., 2004).

4.4 | Differences in grassland responses and wider implications

The community composition of the limestone grassland was generally more responsive to eCO_2 than the acidic grassland, potentially due to its communities comprising a much larger proportion of sedges, the most CO_2-responsive PFT. Differences in soil conditions between the grasslands may have facilitated such a strong response to eCO_2 in the limestone grassland. As mentioned above, sedges are effective at liberating P from inorganic P forms (Lambers et al., 2008), and potential mineral P sources in the limestone soil include the calcium phosphate mineral apatite, which is readily weathered by organic acid anions (Mendes et al., 2021). In contrast, in the acidic grassland, the dominant forms of inorganic P are likely to be bound in highly recalcitrant iron (Fe) and aluminium (Al) phosphate (Gérard, 2016). Furthermore, of the two sedge species that occur in the acidic grassland, one (*C. nigra*) does not form dauciform roots (Davies et al., 1973; Johnson, 2007). Therefore, plants in the acidic grassland are potentially more dependent on biochemical mineralisation of organic P (Johnson et al., 1999; Taylor et al., 2021), while in the limestone grassland, different plants may access a more diverse range of P sources (Phoenix et al., 2020), providing more opportunities to exploit the additional C for P exchange that eCO_2 can provide.

While the relatively young soils in our limestone grassland are very different from the typical highly weathered acidic soils of tropical regions (Lugli et al., 2020), our results may have broader implications for other P-limited ecosystems, especially herbaceous systems. Plant communities that contain a diverse range of P-acquisition strategies, including organic acid exudation, may be especially effective at liberating additional P resources and potentially stimulating productivity (Reichert et al., 2022). For instance, our recent work demonstrates that under eCO_2, limestone grassland productivity increased by 16% with no change in microbial P pools, in stark contrast to the species-poor acidic grassland under eCO_2, where plant productivity and P uptake declined by 11 and 20%, respectively, which was accompanied by a 36% increase in the size of the P pool

immobilised by soil microbes (Keane et al., 2023). Effective mobilisation of soil P from Ca phosphates and organic matter, such as by *Carex* sedges, may be allowing plants to successfully compete with microbes under eCO_2. Consequently, grassland communities comprising a large proportion of plants with adaptations that allow for extensive mobilisation of soil P may respond positively to a high CO_2 future.

It is possible that the high cover dominance of grasses in the acidic grassland (70%–95%) and their dense light-excluding canopy (Hautier et al., 2009) could have constrained the eCO_2 responses of other PFTs, and to a greater extent than in the more functionally even limestone grassland. As plant responses to eCO_2 can be highly species-specific (Hanley et al., 2004; see also Figures S4 and S7), the more speciose limestone grassland may have a greater likelihood of containing eCO_2-responsive species and thus be more responsive at the community level (Niklaus et al., 2001). With more species to respond and a more balanced competitive landscape in the limestone grassland, eCO_2 may drive greater changes in individual fitness and interspecific interactions that lead to shifts in community and functional composition. If this is the case, more species-rich grasslands, and possibly other ecosystems, may be more responsive to future CO_2 concentrations than their species-poor counterparts (Kleynhans et al., 2016; Niklaus et al., 2001).

5 | CONCLUSIONS

We have shown that elevated CO_2 concentrations, nutrient inputs and the interactions between these biogeochemical drivers can have substantive effects on the community and functional composition of the two P-limited grasslands. After only 3 years of CO_2 fumigation, the community composition of both grasslands and the PFT composition of the limestone grassland changed significantly. In the latter case, a strong positive response of *Carex* sedges to eCO_2 meant sedges surpassed co-occurring grasses as the dominant PFT when eCO_2 was combined with N application. Elevated CO_2 and P interacted to significantly increase legume abundance. Long-term N addition significantly reduced richness in both grasslands, and P reduced diversity in the limestone grassland, with both nutrients restructuring communities toward graminoid dominance.

We propose that novel plant communities resulting from CO_2–nutrient interactions in the limestone grassland may reflect changes in the effectiveness of plant P acquisition and subsequent competition for P as N additions increase P demand and eCO_2 subsidises the carbon cost associated with acquiring P. We show that the limestone grassland communities are more susceptible to eCO_2 and its nutrient interactions than their acidic counterparts. This may be due to: (1) its more diverse species assemblage creating more opportunities to exploit novel conditions; (2) a greater proportion of CO_2-responsive species; and (3) soil conditions

being more conducive to benefitting from carbon-intensive P-acquisition strategies.

We conclude that two globally pervasive biogeochemical perturbations, elevated CO_2 concentrations and nutrient pollution, can substantively affect the community and functional composition of P-limited grasslands. Crucially, stronger responses may be seen in more diverse plant communities harbouring a range of P-acquisition strategies, and interactions between eCO_2 and nutrient pollution could create novel plant communities with currently unknown consequences for the functioning of grassland ecosystems.

AUTHOR CONTRIBUTIONS

Christopher R. Taylor: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; validation; visualization; writing – original draft; writing – review and editing. **Luke C. England:** Investigation; writing – review and editing. **J. Ben Keane:** Conceptualization; methodology; supervision; writing – review and editing. **Jessica A. C. Davies:** Methodology; supervision; writing – review and editing. **Jonathan R. Leake:** Conceptualization; methodology; writing – review and editing. **Iain P. Hartley:** Conceptualization; funding acquisition; methodology; writing – review and editing. **Simon M. Smart:** Methodology; writing – review and editing. **Victoria Janes-Bassett:** Methodology; supervision; writing – review and editing. **Gareth K. Phoenix:** Conceptualization; funding acquisition; methodology; project administration; supervision; writing – original draft; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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