

RESEARCH ARTICLE

Species interactions amplify functional group responses to elevated CO₂ and N enrichment in a 24-year grassland experiment

Neha Mohanbabu¹  | Forest Isbell²  | Sarah E. Hobbie²  | Peter B. Reich^{1,3} 

¹Department of Forest Resources, University of Minnesota, St. Paul, Minnesota, USA

²Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, Minnesota, USA

³Institute for Global Change Biology and School for Environment and Sustainability, University of Michigan, Ann Arbor, Michigan, USA

Correspondence

Neha Mohanbabu, Department of Forest Resources, University of Minnesota, 1530 Cleveland Avenue North, St. Paul, MN 55108, USA.
Email: nehamohanbabu@gmail.com

Funding information

Biological and Environmental Research, Grant/Award Number: DE-FG02-96ER62291; Directorate for Biological Sciences, Grant/Award Number: NSF-DBI-2021898; Division of Environmental Biology, Grant/Award Number: DEB-0322057, DEB-0620652, DEB-1120064, DEB-1234162, DEB-1242531, DEB-1753859, DEB-1831944 and DEB-2224854

Abstract

Plant functional groups (FGs) differ in their response to global changes, although species within those groups also vary in such responses. Both species and FG responses to global change are likely influenced by species interactions such as inter-specific competition and facilitation, which are prevalent in species mixtures but not monocultures. As most studies focus on responses of plants growing in either monocultures or mixtures, but rarely both, it remains unclear how interspecific interactions in diverse ecological communities, especially among species in different FGs, modify FG responses to global changes. To address these issues, we leveraged data from a 16-species, 24-year perennial grassland experiment to examine plant FG biomass responses to atmospheric CO₂, and N inputs at different planted diversity. FGs differed in their responses to N and CO₂ treatments in monocultures. Such differences were amplified in mixtures, where N enrichment strongly increased C3 grass success at ambient CO₂ and C4 grass success at elevated CO₂. Legumes declined with N enrichment in mixtures at both CO₂ levels and increased with elevated CO₂ in the initial years of the experiment. Our results suggest that previous studies that considered responses to global changes in monocultures may underestimate biomass changes in diverse communities where interspecific interactions can amplify responses. Such effects of interspecific interactions on responses of FGs to global change may impact community composition over time and consequently influence ecosystem functions.

KEYWORDS

BioCON, FACE experiment, herbaceous, long-term, resource addition, temperate grassland

1 | INTRODUCTION

The impact of global change on plants can vary depending on their inherent physiological and functional differences and may be further influenced by interspecific interactions (Reich et al., 2004). Several manipulative experiments have shown changes in plant functional

group (FG) and species composition under various environmental changes (Avolio et al., 2014; Griffin-Nolan et al., 2019; Gruner et al., 2017; Ladouceur et al., 2022; Polley et al., 2003; Reich, 2009; Zavaleta et al., 2003), suggesting differential responses of various groups. Additionally, these shifts in composition were more likely under multiple simultaneous resource changes (Avolio et al., 2021)

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and in studies spanning multiple decades (Komatsu et al., 2019). But long-term temporal trends in responses of different FGs to global change factors and the extent to which species interactions modify those responses (Collins et al., 2022) are less well-understood. Long-term biodiversity experiments crossed with global change treatments offer a unique opportunity to study the interactive effects of global change treatments on biomass and how they depend on plant interspecific interactions (hereafter species interactions). Understanding the potentially complex influence of environmental change and species interactions on long-term responses of plants is more pertinent than ever given ongoing anthropogenic influence on biogeochemical cycles.

Plant FGs will likely respond differently to global changes that constitute resource addition because of inherent differences in resource acquisition and use (Figure 1). In monocultures, all FGs may respond positively to global changes that constitute resource enrichment, as most plants are limited by low-resource supply rates (Bloom et al., 1985; Elser et al., 2007; Farrior et al., 2013; Fay et al., 2015). But, given differences between C3 and C4 plants in their photosynthetic physiology and N and water use efficiency (Knapp, 1993;

Pearcy & Ehleringer, 1984), herbaceous C3 plants (grasses, legumes, and non-leguminous forbs [hereafter 'forbs']) may respond more strongly to N and/or CO₂ enrichment than C4 grasses (Reich, Tilman, et al., 2001; Wand et al., 1999) (but see Reich et al., 2018). Similarly to C4 grasses, legumes may also respond weakly to N enrichment as they are usually not limited by N availability. Such responses of plants growing in monocultures provide a baseline for determining the impact of global changes in the absence of plant interspecific interactions (i.e., direct response to an environmental change), whereas responses in mixtures reflect simultaneously responses to environmental change and to interspecific interactions (which may themselves change under global change treatments, Collins et al., 2022). Therefore, comparisons of responses between monocultures and mixtures can be used to quantify the impact of species interactions on responses to experimental treatments, as illustrated by other ecological studies (Loreau, 2010; Mas et al., 2024; Spehn et al., 2002; Vandermeer, 1981; Wright et al., 2017).

Species interactions may strongly influence responses of FGs to global change drivers under mixture conditions (Figure 1). For example, the hypothetical competitive advantage of C3 plants in elevated

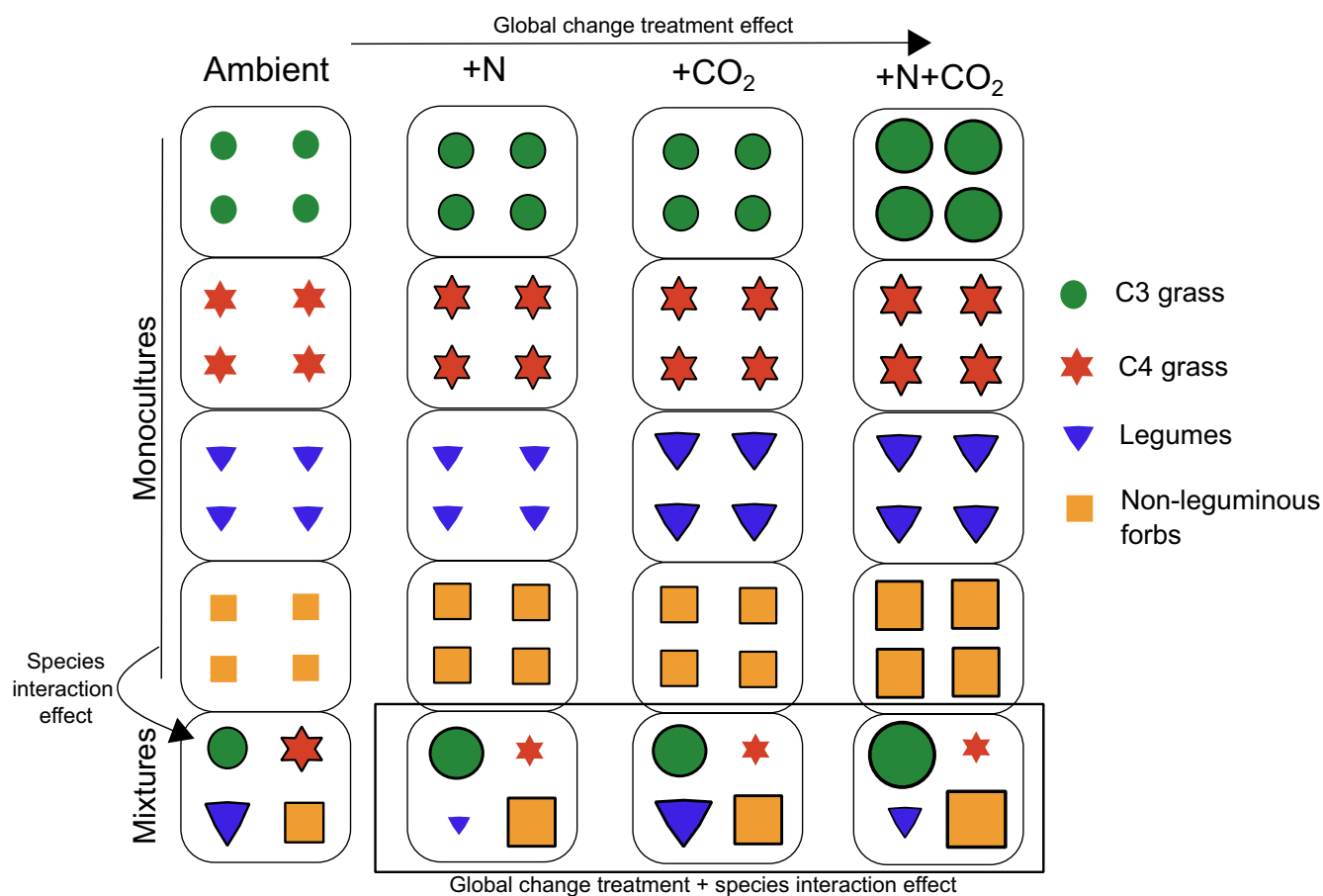


FIGURE 1 Conceptual figure showing expectations under different global change treatments. The shapes denote different functional groups (FGs), the size of the symbols indicate whether a particular group increases or decreases (in terms of biomass) in response to the different treatment conditions. Note that in the experiment, the change in FG response could be due to changes in size and/or number of individuals. All experimental plots are the same size but biomass measures were standardized for planted proportions in mixtures. The figure shows trends for monocultures and 16-species mixtures (for simplicity) where the planted proportion for each FG is 1/4th of the planted proportions in monocultures. A summary of these hypotheses and whether the results matched the predictions is included in Table S1.

N and/or CO₂ supply may be more pronounced in the presence of other species that survive better in low N and/or CO₂ supply (sensu R* competition theory, Tilman, 1982). Thus, C3 grasses and forbs may show a stronger positive response than C4 grasses to N (or CO₂) addition when growing with other FGs due to reduced intra-specific competition compared with while growing in monocultures (because interspecific competition is weaker than intraspecific competition, Adler et al., 2018). Legumes, on the other hand, may be competitively superior under low N conditions due to their biological N fixation (Lee, Tjoelker, et al., 2003), and experimental N addition may reduce legume abundance, especially when they are growing with non-legumes, due to increased interspecific competition under high-N versus low-N conditions (Ren et al., 2016; Suding et al., 2005; Tognetti et al., 2021). Finally, C4 grasses, which often have high N use efficiency and comparatively low requirements for CO₂ (Ghannoum et al., 2011; Pearcy & Ehleringer, 1984), may benefit less than other FGs at higher availability of N and/or CO₂, and thus perform poorly when grown with other species, resulting in a negative response to N and/or CO₂ in mixture. In general, we posit that the FGs that respond most positively in monoculture to elevated CO₂ and/or enriched N will also respond positively to those treatments in mixture, but those that respond less positively in monoculture may instead respond neutrally or negatively in mixture, as they may be outcompeted by other FGs that are more favored by the global changes. If so, then species interactions in mixtures would tend to amplify the difference in FG group responses to global changes between monocultures and mixtures.

These hypothetical differences in FG responses to global change drivers in mixtures versus monocultures may also vary over time (Smith et al., 2009) and change with global change treatments (Collins et al., 2022), but expectations for these dynamics remain elusive given numerous pathways for change and the limited number of relevant long-term studies to date. The magnitude of global change effects may diminish or grow over time regardless of positive or negative initial responses. A dampening temporal effect may arise, for example, from physiological acclimation of plants to elevated resources (Warren et al., 2015) or effects on other abiotic factors that reduce availability of other resources (Borer et al., 2014; Kimmel et al., 2020; Lawes & Gilbert, 1880; Yue et al., 2019). In contrast, the effect of global change factors may increase over time if effects on abiotic factors increase supply of other resources. For example, in the same long-term experiment reported here, elevated CO₂ had a stronger positive effect on pure C4 grass plots in the later years of the experiment due to increased net N mineralization from elevated CO₂ in those C4 communities (Reich et al., 2018). Alternatively, effects may remain the same over time or show large year-to-year variation driven mainly by interactions with environmental parameters such as precipitation and temperature (Avolio et al., 2020). Additionally, diversity loss either over time or in response to treatments (Isbell et al., 2013) may alter species interactions that may influence FG responses. For instance, declining relative abundance of legumes over time may indirectly impact the response of other C3 species to global change treatments by reducing soil N supply

(Pirhofer-Walzl et al., 2012; Spehn et al., 2002). Such possibilities highlight the need to study temporal trends of FG responses to environmental change under both monoculture and mixture conditions.

To address this need, we examined the differential responses of herbaceous FGs to global environmental change factors in monocultures, without species interactions, and in mixtures where interspecific interactions occurred. We used data from a 24-year biodiversity and global change grassland experiment, BioCON, in Minnesota, USA, to first examine whether interactions among FG change in response to global change treatments and to specifically examine whether species interactions amplify the difference in FG responses to global change treatments between monocultures and mixtures. Additionally, we explored temporal trends to assess if these FG responses diminish or grow over time.

2 | MATERIALS AND METHODS

Experiment design: The BioCON experiment, which manipulates Biodiversity, N and CO₂, was started in 1997 at Cedar Creek Ecosystem Science Reserve (East Bethel, MN, USA, 45°40'N, 93°18'W), a Long-Term Ecological Research site. The compositionally neutral full-factorial experiment comprised 296 plots that varied in their initial planted diversity (1, 4, 9 or 16 species), N (ambient or +4 g m⁻² year⁻¹) and CO₂ (ambient or +180 ppm) treatments applied in a split-plot design for the CO₂ treatment (Reich, Knops, et al., 2001). The diversity gradient was created by experimentally assembling plant communities with one species, random selection of four or nine species and/or all 16 species. As 36 of the nine species plots have since been used for a sub-experiment involving temperature and drought treatments, we excluded those plots from our analyses leaving 260 plots. The species were chosen to represent common or naturalized prairie species in the region and spanned four FGs: C₃ grasses (*Bromus inermis*, *Elymus repens* [formerly *Agropyron repens*], *Koeleria macrantha* [formerly *Koeleria cristata*], and *Poa pratensis*), C₄ grasses (*Andropogon gerardii*, *Bouteloua gracilis*, *Schizachyrium scoparium*, and *Sorghastrum nutans*), legumes (*Amorpha canescens*, *Lespedeza capitata*, *Lupinus perennis*, and *Petalostemum villosus*), and non-legume forbs (*Achillea millefolium*, *Anemone cylindrica*, *Asclepias tuberosa*, and *Solidago rigida*). Since the species composition was randomly chosen for the four and nine species plots, each replicate for these two diversity levels differs in species and FG composition. Species found in a plot other than those originally planted there were weeded annually. Additionally, to maintain the study site in a grassland state, the plots were burnt in spring for half of the years between 2000 and 2012 (Adair et al., 2009) and every fall since 2013.

2.1 | Plant biomass

Late in each growing season in August in the majority of plots in almost all years, aboveground biomass in a pre-marked 10×100 cm

strip was clipped and sorted at the species level. The part of the plot from which biomass data were collected shifted annually to reduce potential influence of clipping on a single part of the plot. Species-specific cover data from a constant predetermined and never clipped 50×100cm region of the plot were also recorded for every plot each year (except for a subset of four species plots during 2020 as all efforts were scaled down due to the pandemic). Using cover and biomass data, we then generated species-specific correlations between the two to estimate biomass data for all years based on cover data. This step allowed us to (a) impute biomass values when only cover data were available; and (b) obtain a more representative estimate of biomass for the plot as cover data spanned a larger area of the plot compared to clip-strip biomass data. The data and code are available on Dryad (Mohanbabu et al., 2024).

2.2 | Statistical analysis

All statistical analyses were performed in R version 4.2.2 (R Core Team, 2022).

2.2.1 | Diversity–interaction models

The diversity–interaction (DI) modeling framework was developed to better understand the contributions of species identity and interaction (i.e., interspecific interaction) effects on ecosystem function (Connolly et al., 2013; Kirwan et al., 2009). It encompasses a series of increasingly complex models with total aboveground biomass as the dependent variable, species identity effects (i.e., providing estimates for monocultures or more generally, testing how changing initial proportions of a species impact plot biomass) and/or species interaction effects (i.e., testing whether and how much interspecific interactions in mixtures alter biomass as a deviation from monoculture expectations) and other experimental treatment variables as the independent variables. For an experiment such as ours with 16 species, independent variables can accumulate quickly if the effects of all pairs of species are considered (Kirwan et al., 2009). We chose to simplify the models by considering FG identity and interaction effects rather than species-level effects. Additionally, the model framework also allows evaluation of interaction effects with varying degrees of complexity. The most complex model, “separate pairwise interaction model,” assumes that each pairwise FG interaction is unique and an additional independent variable (i.e., 6 variables in total) is included in the model for each pairwise combination. A simplified version of the model is the “additive FG-specific interaction model,” which assumes that each FG differs in its interaction, but the interaction is independent of the identity of the interacting FG (i.e., C3 grass interacts similarly with C4 grass, legumes and forbs). The model includes four additional variables for each FG. A further simplification of the model is the “average interaction effects model,” which assumes that pairs of FGs interact similarly regardless of their identity. This model includes one additional independent variable of

an average effect of all pairwise interactions. The simplest model, “FG identity model,” assumes there are no interactions between FG. We fit the five different DI models using the DImodelsMulti package (Byrne et al., 2024) and used likelihood ratio tests and AICc values to determine the most parsimonious model. All models included the N and CO₂ treatments as the “extra_fixed” term in the model. To account for the repeated measures in a split-plot experimental design, we specified a compound symmetry temporal correlation structure. We log-transformed total aboveground biomass to meet normality and heteroskedasticity assumptions. The “average interaction effect” or the “AV” model was the most parsimonious model suggesting that assuming similar interaction strengths between FGs is a reasonable approximation of the system. However, we acknowledge that there might be some pairwise differences between different FGs that were not readily detectable with our sample sizes but include those results in supplemental information (Figures S1 and S2). The significant AV interaction effect also indicates that species interactions significantly alter the total biomass in the plots. An additional strength of this method is that it accounts for the net of both positive and negative interactions among FGs.

2.2.2 | Functional group responses to global changes

Because of the large number of species, presenting, interpreting, and discussing the results for each species in monocultures versus mixtures, and in response to different global changes over time is beyond the scope of this study. Instead, for the sake of simplicity and potential for generalization, we present results for each of the FGs.

FG-specific biomass was standardized for planted proportions in the mixtures by dividing estimated biomass by planted FG proportions. This was necessary as the four and nine species plots varied in their planted FG proportions unlike the 16-species plots where each FG was planted to 25% of the total seed-mass or the monocultures where FG was either 0% or 100% of the plot. For example, if C3 grasses in a 9-species plot were planted at 22.2% (i.e., 2 out of 9 species was a C3 grass), then we standardized the biomass of C3 grass for the plot by dividing the biomass by 0.222. This allowed us to compare biomass of C3 grasses across plots with different initial planted proportions.

We then use a linear mixed-effects model (nlme) (Pinheiro et al., 2023) with log-transformed FG-specific biomass as the dependent variable, and log-transformed species richness, N and CO₂ treatments, FG identity (ID), experiment year, all their interactions as fixed effects and plot nested within ring as a random effect to account for the split-plot experimental design. Additionally, the nlme model uses an autoregressive component to account for temporal autocorrelation in repeated measurements. Log-transforming the FG specific biomass accounts for proportional responses of FG biomass rather than just a diversity effect on biomass. The two-way and three-way interactions including FG ID, species richness, and global change treatments can be used to understand FG responses

to the different treatments. However, using biomass at the FG level for each plot results in many 0s as some of the groups have become locally extinct. To confirm that our model results were not greatly influenced by violations of the assumptions, we repeated the analyses using average values across plots for each treatment and year combination (Table S3). The results from both those models are largely similar and we present the latter results in the supplemental information.

All the models treated the four diversity levels as a continuous variable but for the purposes of data visualization in the manuscript, we present the results for mixtures as an average value across the 4, 9, and 16 species plots (weighted equally) and include more detailed figures in the supplemental information (Figures S3 and S4). This allows us to focus on the trends that were largely consistent across the diversity levels while acknowledging the complexity that arises with varying species composition and richness.

3 | RESULTS

3.1 | Influence of global change treatments on FG interactions

The DI models indicate that interactions between FG had a significant effect on the total biomass (Figure 2), which is observed as larger biomass for all FG in mixtures compared to monocultures (Figure 3). The most likely model further suggests that an average interaction term (i.e., similar net interactions among all pairs of FGs) is sufficient to explain various FG interactions that occurred in diverse plots. The average interaction effect always had a positive impact on above-ground biomass and increased over time in all treatments (Figure 2),

which resulted in greater total biomass in mixtures compared with monocultures. Global change treatments did not strongly influence the average interaction effects in this experiment (Treatment, $p = .17$; Treatment $\times$ Year, $p = .10$). Thus, in presenting the results, we emphasize the presence of significant interaction between pairs of FGs, which may influence plant responses in mixtures.

However, it is likely that FGs or pairs of FGs varied in their interactions over time and in response to global change treatments, albeit not statistically significantly. The interaction strengths of C3 grasses strengthened over time especially under N addition (Figure S1) likely driven by increasing pairwise interaction between C3 and C4 grasses, which show the steepest temporal trends (Figure S2). In contrast to C3 grasses, the strength of legumes modestly declined under N addition (Figure S1), a trend that is clearly evident for interactions between legumes and forbs which are significant and increasing over time under ambient N but insignificant under N addition treatments (Figure S2).

3.2 | Effect of N enrichment on FG biomass

As expected, FGs differed in their responses to global change treatments, but in complex ways (Figure 3; Figure S3). The effect of N on FG absolute biomass depended on FG identity (Table S2, N $\times$ FuncGrp $p < .0001$), differed with planted species richness (Table S2, $\log(\text{SpRich}) \times \text{N} \times \text{FuncGrp}$ $p = .0002$), and also varied over time (Table S2, $\log(\text{SpRich}) \times \text{N} \times \text{FuncGrp} \times \text{Year}$ $p = .09$). As hypothesized, N enrichment increased C3 grass biomass, whereas it decreased legume biomass, especially at high levels of plant diversity. The responses of C4 grass and forb biomass to N were more variable and depended on the plant diversity level. Additionally, the positive effect of N on biomass decreased over time for C3 grasses while

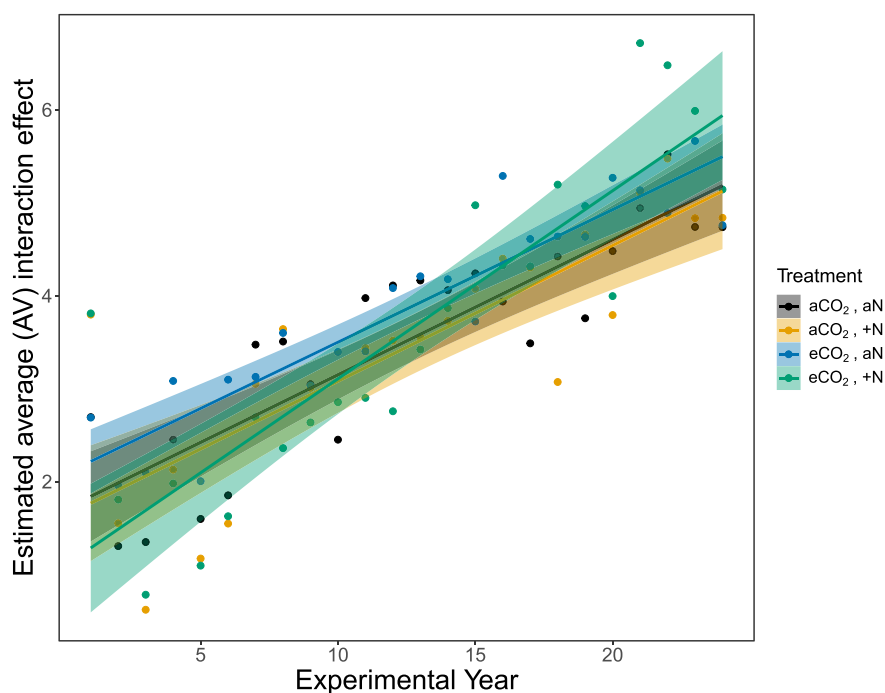


FIGURE 2 Estimates of the interaction effect based on the most parsimonious diversity-interaction model (average [AV] interaction effect model). The model suggests that the strength of the different pairwise functional group interactions are statistically similar, and an average value can be used to predict total biomass. The units of AV are in log grams of biomass/m². "a" indicates ambient conditions and "e" or "+" indicate enriched conditions. The shaded regions are the 95% confidence intervals.

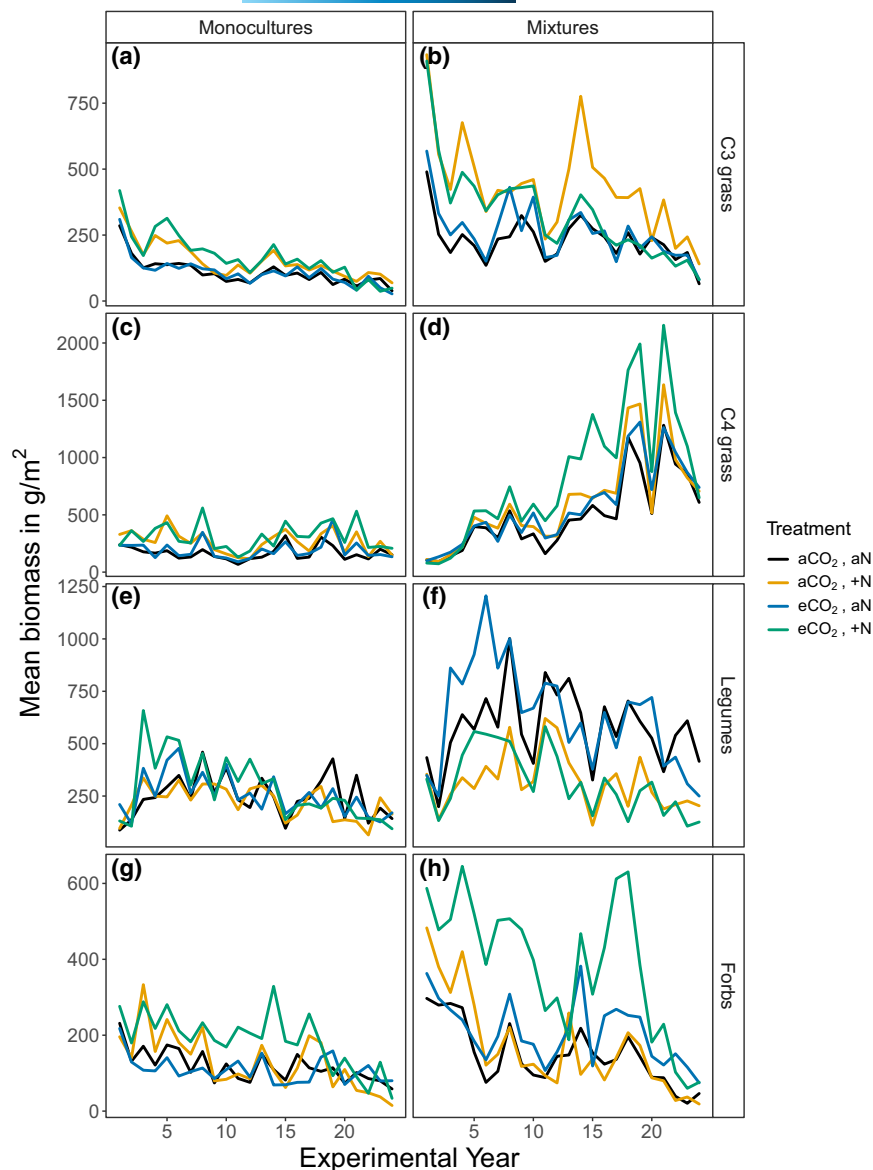


FIGURE 3 Temporal variation in aboveground peak biomass (g/m^2) in monocultures and mixtures for (a, b) C3 grasses, (c, d) C4 grasses, (e, f) Legumes, and (g, h) Forbs, respectively under the different treatment conditions. Biomass for mixtures is averaged across the 4, 9, and 16 species plots with each diversity level weighted equally. Note that the y-axis range varies for the different FGs. "a" indicates ambient conditions and "e" or "+" indicate enriched conditions. See Figure S3 for responses at all four diversity levels.

the temporal patterns for the other FGs were more idiosyncratic such that patterns were specific to each richness level (Figure 4; Figure S4, left column).

3.3 | Effect of elevated CO_2 on FG biomass

The effect of CO_2 on FG absolute biomass also depended on FG identity (Table S2, $\text{CO}_2 \times \text{FuncGrp}$ $p < .0001$) and species richness (Table S2, $\log(\text{SpRich}) \times \text{CO}_2 \times \text{FuncGrp}$ $p < .0001$) and varied over time (Table S2, $\log(\text{SpRich}) \times \text{CO}_2 \times \text{FuncGrp} \times \text{Year}$ $p = .05$). Increasing species richness amplified the positive effect of CO_2 on legumes and C4 grass biomass compared with monocultures. Forb biomass responded positively to CO_2 but only at high diversity levels. In contrast, and in opposition to expectations, elevated CO_2 did not influence C3 grass biomass. The responses of C4 grasses and legumes to CO_2 also showed strong temporal trends; the effect of CO_2 was increasingly positive for C4 grasses and was positive

initially and switched to negative in the more recent years for legumes. C3 grasses and forbs did not show significant temporal trends in response to CO_2 (Figure 4; Figure S4, middle column).

3.4 | Interaction of N enrichment and elevated CO_2 on FG biomass

The responses of FG biomass to enriched N or elevated CO_2 at different species richness levels was often dependent on the presence or absence of the other global change treatment (Table S2, $\log(\text{SpRich}) \times \text{N} \times \text{CO}_2 \times \text{FuncGrp}$, $p < .001$). For instance, the positive CO_2 effect on biomass for C4 grasses was consistently larger under enriched N rather than ambient N (Figure 4e,f). Similarly, the effect of CO_2 on biomass of C3 grasses and forbs was more positive under enriched N, at least in the initial years of the experiment (Figure 4b,c,k,l). Additionally, the negative N effect on legumes was larger under elevated than ambient CO_2 , especially for some richness levels.

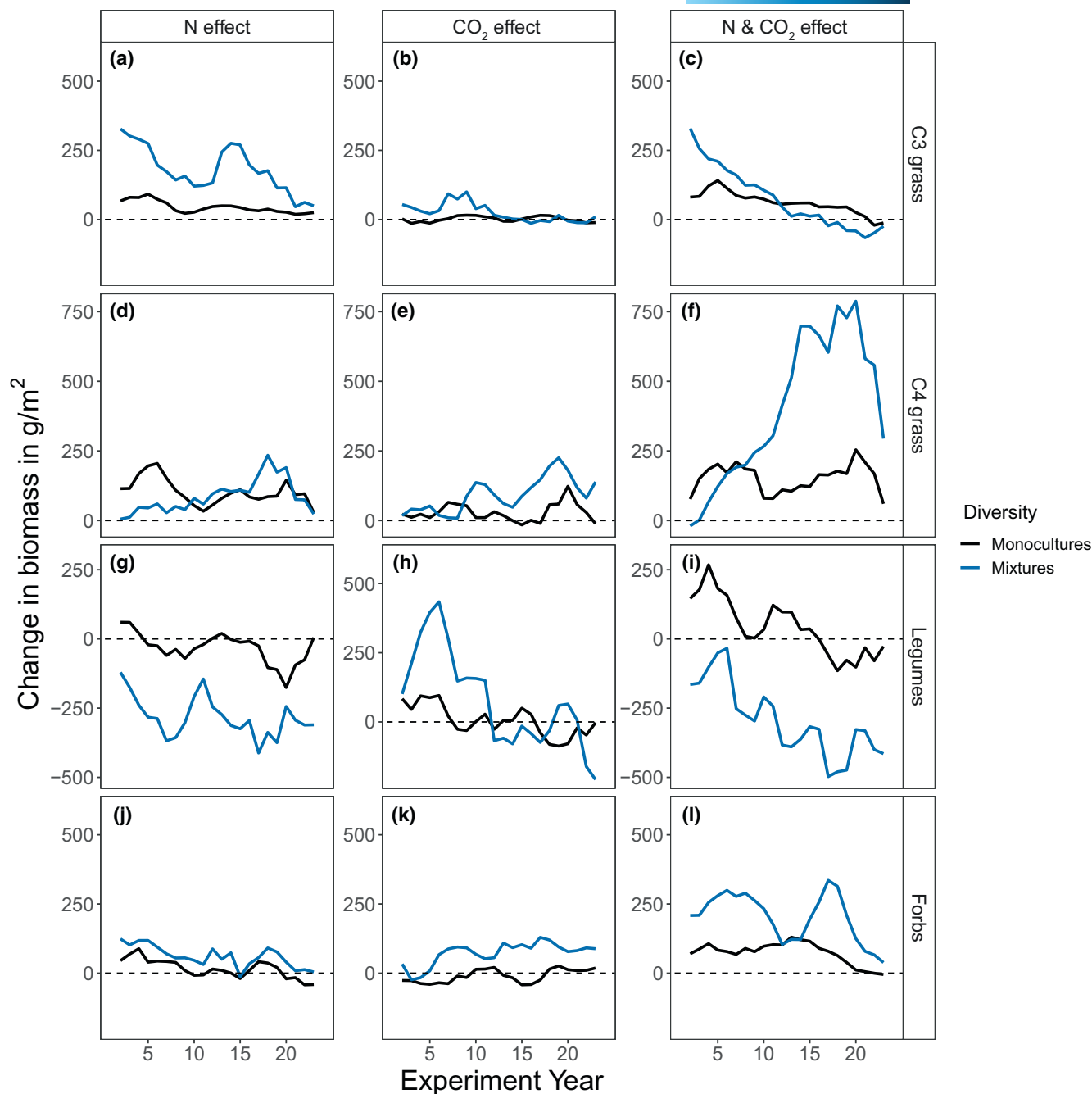


FIGURE 4 Effect of N, CO₂, and N and CO₂ on (a–c) C3 grasses, (d–f) C4 grasses, (g–i) Legumes, and (j–l) Forbs for monocultures and mixtures (average across 4, 9, and 16 species plots weighted equally). Effect was calculated for each FG×diversity combination as the biomass in treatment—biomass under ambient N and ambient CO₂ conditions. The lines pass through points that are moving averages centered at the middle of 3-year windows. The y-axis is scaled such that the range width is the same but the minimum and maximum values may differ (i.e., the magnitude is comparable across graphs even though the actual axis varies). See [Figure S4](#) for responses of 4, 9, and 16 species plots and [Figure S5](#) for proportional responses.

3.5 | Influence of species interactions on FG response to global change treatments

In general, species interactions (i.e., interspecific interactions) amplified the impacts of global change treatments on FG group absolute biomass responses in mixtures compared with monocultures ([Figure 5](#)). As hypothesized, FGs that responded positively

to enriched N or elevated CO₂ in monocultures often responded even more strongly under mixture conditions. For example, C3 grasses, which responded positively to N under both ambient and elevated CO₂ in monocultures, also showed a strong positive effect of N in mixtures, especially in the initial years of the experiment ([Figure 4](#), row 1). C4 grasses too showed strong positive responses to N and CO₂ addition in monocultures that

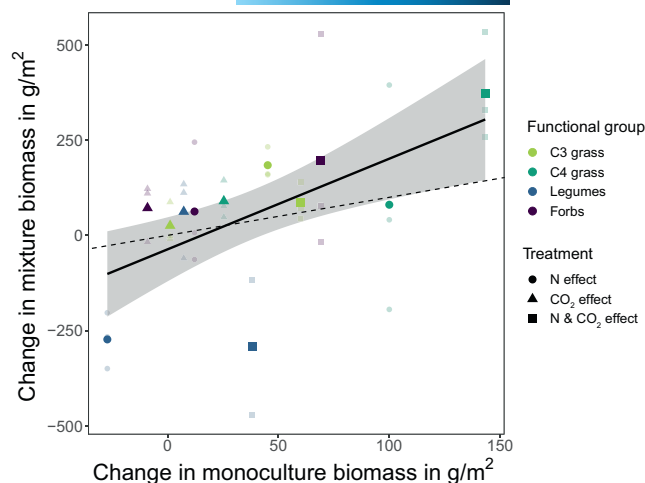


FIGURE 5 Response to global change treatments of FG biomass in mixtures (4, 9, and 16 species) in relation to monoculture response to the treatments. Response (or change) is the average over all years of difference between plant biomass in a treatment-plant biomass in ambient condition. The darker, larger points are average values for mixtures and the lighter, smaller points show averages for 4, 9 and 16 species treatments separately. The line is fit through the values for 4, 9 and 16 species and the relationship was statistically significant with slope = 2.38 (.68), $p = .001$ and $R^2 = .24$. The dashed line is the one-to-one line, and the shaded regions are the 95% confidence intervals.

were further amplified under mixture conditions (Figure 4f). On the other hand, FGs that showed a neutral or negative response to treatments under monocultures were more likely to respond more negatively in mixtures. This was evident in the effect of N on biomass of legumes and forbs (Figure 4g,j). However, there were exceptions to this generalization as can be seen by the negative effect of N on C4 grasses (Figure S4d) and positive effect of N on forbs (Figure S4j) in 9-species plots and the lack of a strong response of C3 grasses in mixtures to CO_2 (Figure 4b), all of which highlight the complexity of the influence of species interactions on responses of FGs to global change factors.

4 | DISCUSSION

Herein, using a unique long-term biodiversity and global change manipulation experiment, we showed that multiple global change treatments impacted different perennial herbaceous plant FGs biomass responses in an interactive manner (Figures 3 and 4). Additionally, those responses were sensitive to species interactions (i.e., interspecific interactions) and showed strong temporal patterns. In most cases, the responses of FGs to N or CO_2 manipulation depended on the identity of the FGs and planted diversity of the community, and also varied over time. Importantly, species interactions (i.e., inter-FG in addition to intra-FG and intraspecific competition) in mixtures amplified the difference in FG response to global changes between monocultures and mixtures (Figure 5), but the temporal trends were variable and depended on the FG identity and diversity level. Our

results underscore the complex influence of species interactions on plant response to global changes and suggest that past studies conducted at the individual plant or population level may have underestimated the effects of global change on plant FGs.

4.1 | Effect of N enrichment on FG biomass

Responses of FGs to N enrichment were group-specific and varied with species richness and CO_2 treatment. C3 grasses responded positively to N under both ambient and elevated CO_2 for both monocultures and mixtures, thus confirming long-standing predictions that C3 plants should respond positively to elevated CO_2 and/or enriched N (Reich, Knops, et al., 2001; Wedin & Tilman, 1996; Zeng et al., 2010). However, the positive effect has diminished over the years. One potential explanation for this decline is soil acidification under N enrichment relative to ambient conditions resulting in colimitation of resources for C3 grasses (Tian & Niu, 2015). Alternatively, increasing ambient temperature over the years (Figure S6) may have negatively affected the cool-season C3 grasses.

In contrast to C3 grasses, legumes responded negatively to N enrichment especially under mixture conditions. As biological N-fixers, it is likely that they lack competitive advantage under high soil N availability (Lee, Reich, et al., 2003) and therefore decreased in biomass, similar to other experimental and observational studies (Pennings et al., 2005; Ren et al., 2016; Suding et al., 2005; Tognetti et al., 2021; Vázquez et al., 2022).

Contrary to our expectations based on physiological traits of C4 species (Percy & Ehleringer, 1984), N enrichment had a positive effect on C4 grasses especially in low diversity plots (1- and 4-species). But the responses of C4 grasses in high diversity plots were more variable; N enrichment initially increased and then decreased C4 grass biomass in 9-species plots, whereas it initially decreased and then modestly increased C4 grass biomass in the 16-species plots.

4.2 | Effect of CO_2 enrichment on FG biomass

Elevated CO_2 had only a modest effect on C3 grasses, a positive effect on C4 grasses and non-leguminous forbs, and a temporally variable effect on legumes. The lack of a strong positive effect of CO_2 on C3 grasses is contrary to our initial expectations. Even though elevated CO_2 may have reduced photorespiration in C3 plants, their biomass may have been co-limited by other resources or temperature, similar to total biomass (Reich et al., 2014, 2020). However, the positive response of C3 non-leguminous forbs to elevated CO_2 indicated that at least some C3 species did benefit from elevated CO_2 .

Interestingly, CO_2 had an increasingly positive effect on C4 grasses even under (and more so in) mixtures. This is consistent with findings from monoculture and 4-species C4 grass plots from the same experiment (Reich et al., 2018) but mechanisms through which elevated CO_2 influenced C4 species in mixed FG communities are yet unknown.

Additionally, legumes responded positively to CO₂ in the initial years of the experiment as reported in earlier work at BioCON (Lee, Tjoelker, et al., 2003), likely due to enhanced biological N fixation that may have alleviated N-limitation. Over time, legume response to CO₂ declined, potentially an outcome of increased limitation by soil nutrients other than N such as phosphorus which may directly limit plant growth, or molybdenum which may reduce N-fixation and thus indirectly limit plant growth (Hungate et al., 2004; van Groenigen et al., 2006). Furthermore, previous research from the same study system has shown that legume species may differ in the magnitude of response to global change treatments (West et al., 2005). Such species-specific differences in response to CO₂ may explain some of the temporal trends if accompanied by changing composition of legumes in the experimental plots over time.

Lastly, the responses of FGs to elevated CO₂ were not consistent with the predictions based on earlier studies (Table S1). Therefore, these long-term results likely help fill important knowledge gaps, while expanding the dimensions of those gaps, in our understanding of plant FG responses to CO₂.

4.3 | Effect of N and CO₂ enrichment on FG biomass

The FG responses to the combination of both enriched N and elevated CO₂ were strongly positive for C4 grasses and strongly negative for legumes, whereas C3 plants showed only a modest response (except for forbs in 4-species plots). Even though C4 grasses are not expected to respond strongly to N or CO₂ availability, our results from mixture plots are consistent with previous research in BioCON that showed a strong positive effect of CO₂ on biomass of C4 plants growing in monocultures or solely C4 mixtures (Reich et al., 2018). The mechanism suggested in that study, increased net N mineralization under CO₂, does not likely explain this strong response in mixtures as any change in net N mineralization should also positively impact other FGs. Thus, future work should focus on elucidating proximate mechanisms resulting in positive effects of CO₂ on C4 grasses.

Strikingly, species interactions in mixtures amplified the effect of the global change factors on FGs compared to when they grew in monocultures. Generally, as we hypothesized, FGs that responded positively to the treatments in monocultures were more likely to respond strongly positively under mixture conditions, whereas FGs that responded neutrally or negatively in monocultures were more likely to respond negatively in mixtures (Figure 5). This suggests that FGs that are more responsive in monocultures may benefit from ongoing global changes, but the generality of this needs to be confirmed in other experiments and systems. Even in this study there were exceptions to this generalization: N enrichment had a negative effect on C4 grasses and a positive effect on forbs in 9-species mixtures despite opposite effects in monocultures. Furthermore, the effect of diversity on FG biomass response to global change treatments did not always increase monotonically with richness as

responses from 4-species plots were sometimes higher than those of 9-species plots (Figures S3 and S4). Additionally, responses of FGs to treatments in 4- and 9-species plots were more variable, likely due to large variation in initial and subsequent composition among plots (in contrast to 16-species plots that were always seeded identically with all 16 study species). These results highlight the complex influence of species interactions on responses to global change treatments and indicate the potential for underestimation of effects of global change on FG biomass in studies that focus on individual or population levels.

While our results shed light on long-term responses to CO₂ and N of different FGs, the proximate drivers of the temporal trends remain unknown. Over the 24-year period, ambient CO₂ levels have increased almost linearly (Porter et al., 2022), growing season temperatures at the site showed an increasing trend (Figure S6) and rainfall showed non-monotonic inter-annual variation. Similarly, pH and soil solution N, which are proxies for changing abiotic factors in response to treatments, also showed temporal variation, potentially influencing FG responses over time. But these factors were usually only weakly correlated with the effect of treatments on FG biomass (Figure S7). Finally, it is possible that other parameters such as herbivory or disease, may influence some temporal patterns but we unfortunately do not have temporal data to test their effects.

Although intraspecific interactions are important, their relative contribution toward influencing FG responses to global changes remains elusive. The diversity experiment used in this study is best suited for comparing presence and absence of interspecific interactions, using comparisons between monocultures (i.e., no interspecific interactions; only intraspecific interactions) and mixtures (i.e., both interspecific and intraspecific interactions). Future work on experiments with monocultures and mixtures planted at varying densities of different species are needed to tease apart relative contributions of inter- and intra-specific interactions toward FG responses to global changes.

In conclusion, we find that species interactions often amplified the effect of global change on FG responses between monocultures and mixtures. C3 grasses responded more strongly to N enrichment under mixture conditions but the magnitude of this effect diminished over time. In contrast, C4 grasses in mixtures were initially less positively affected by N and CO₂ additions compared to monocultures but switched to an increasingly positive response over time. Lastly, as expected, legumes responded more negatively to N enrichment in mixtures and positively to elevated CO₂, although the latter response was limited to the first few years of the experiment. These results underscore the complex and interactive effects of multiple global change drivers on FG responses in the long-term and highlight the potential pitfalls of extrapolating from responses of FGs in monocultures and/or from short-term experiments.

AUTHOR CONTRIBUTIONS

Neha Mohanbabu: Conceptualization; formal analysis; validation; visualization; writing – original draft; writing – review and editing.

Forest Isbell: Funding acquisition; resources; supervision; writing

– review and editing. **Sarah E. Hobbie**: Funding acquisition; project administration; resources; supervision; writing – review and editing. **Peter B. Reich**: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; writing – review and editing.

ACKNOWLEDGMENTS

We greatly appreciate K. Worm and D. Bahaiddin and many undergraduate interns for assistance with experimental maintenance, data collection, and data curation.

FUNDING INFORMATION

This work was supported by multiple grants over the years: National Science Foundation (NSF) Long-Term Ecological Research (LTER) DEB-0620652, DEB-1234162, DEB-1831944; Long-Term Research in Environmental Biology (LTREB) DEB-1242531 and DEB-1753859; Ecosystem Sciences DEB-1120064; Biocomplexity DEB-0322057, Biodiversity on a Changing Planet (DEB-2224854) and Biological Integration Institutes grant NSF-DBI-2021898 to PBR, SEH, FI. U.S. Department of Energy Programs for Ecosystem Research DE-FG02-96ER62291 to PBR.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data and R code that support the findings of this study are openly available on Dryad at <https://doi.org/10.5061/dryad.9zw3r22q5>.

ORCID

Neha Mohanbabu  <https://orcid.org/0000-0002-6557-131X>

Forest Isbell  <https://orcid.org/0000-0001-9689-769X>

Sarah E. Hobbie  <https://orcid.org/0000-0001-5159-031X>

Peter B. Reich  <https://orcid.org/0000-0003-4424-662X>

REFERENCES

- Adair, E., Reich, P. B., Hobbie, S. E., & Knops, J. M. H. (2009). Interactive effects of time, CO₂, N, and diversity on total belowground carbon allocation and ecosystem carbon storage in a grassland community. *Ecosystems*, 12(6), 1037–1052. <https://doi.org/10.1007/s10021-009-9278-9>
- Adler, P. B., Smull, D., Beard, K. H., Choi, R. T., Furniss, T., Kulmatiski, A., Meiners, J. M., Tredennick, A. T., & Veblen, K. E. (2018). Competition and coexistence in plant communities: Intraspecific competition is stronger than interspecific competition. *Ecology Letters*, 21(9), 1319–1329. <https://doi.org/10.1111/ele.13098>
- Avolio, M. L., Koerner, S. E., La Pierre, K. J., Wilcox, K. R., Wilson, G. W. T., Smith, M. D., & Collins, S. L. (2014). Changes in plant community composition, not diversity, during a decade of nitrogen and phosphorus additions drive above-ground productivity in a tallgrass prairie. *Journal of Ecology*, 102(6), 1649–1660. <https://doi.org/10.1111/1365-2745.12312>
- Avolio, M. L., Komatsu, K. J., Collins, S. L., Grman, E., Koerner, S. E., Tredennick, A. T., Wilcox, K. R., Baer, S., Boughton, E. H., Britton, A. J., Foster, B., Gough, L., Hovenden, M., Isbell, F., Jentsch, A., Johnson, D. S., Knapp, A. K., Kreyling, J., Langley, J. A., ... Tognetti, P. M. (2021). Determinants of community compositional change are equally affected by global change. *Ecology Letters*, 24(9), 1892–1904. <https://doi.org/10.1111/ele.13824>
- Avolio, M. L., Wilcox, K. R., Komatsu, K. J., Lemoine, N., Bowman, W. D., Collins, S. L., Knapp, A. K., Koerner, S. E., Smith, M. D., Baer, S. G., Gross, K. L., Isbell, F., McLaren, J., Reich, P. B., Suding, K. N., Suttle, K. B., Tilman, D., Xu, Z., & Yu, Q. (2020). Temporal variability in production is not consistently affected by global change drivers across herbaceous-dominated ecosystems. *Oecologia*, 194(4), 735–744. <https://doi.org/10.1007/s00442-020-04787-6>
- Bloom, A., Chapin, F. S., & Mooney, H. A. (1985). Resource limitation in plants- an economic analogy. *Annual Review of Ecology and Systematics*, 16, 363–392.
- Borer, E. T., Seabloom, E. W., Gruner, D. S., Harpole, W. S., Hillebrand, H., Lind, E. M., Adler, P. B., Alberti, J., Anderson, T. M., Bakker, J. D., Biederman, L., Blumenthal, D., Brown, C. S., Brudvig, L. A., Buckley, Y. M., Cadotte, M., Chu, C., Cleland, E. E., Crawley, M. J., ... Yang, L. H. (2014). Herbivores and nutrients control grassland plant diversity via light limitation. *Nature*, 508(7497), 517–520. <https://doi.org/10.1038/nature13144>
- Byrne, L., Vishwakarma, R., De Moral, R. A., & Brophy, C. (2024). *DlmodelsMulti: Fit multivariate diversity-interactions models with repeated measures*. R package Version 1.0.
- Collins, C. G., Elmendorf, S. C., Smith, J. G., Shoemaker, L., Szojka, M., Swift, M., & Suding, K. N. (2022). Global change re-structures alpine plant communities through interacting abiotic and biotic effects. *Ecology Letters*, 25(8), 1813–1826. <https://doi.org/10.1111/ele.14060>
- Connolly, J., Bell, T., Bolger, T., Brophy, C., Carnus, T., Finn, J. A., Kirwan, L., Isbell, F., Levine, J., Lüscher, A., Picasso, V., Roscher, C., Sebastia, M. T., Suter, M., & Weigelt, A. (2013). An improved model to predict the effects of changing biodiversity levels on ecosystem function. *Journal of Ecology*, 101(2), 344–355. <https://doi.org/10.1111/1365-2745.12052>
- Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., & Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, 10(12), 1135–1142. <https://doi.org/10.1111/j.1461-0248.2007.01113.x>
- Farrior, C. E., Tilman, D., Dybzinski, R., Reich, P. B., Levin, S. A., & Pacala, S. W. (2013). Resource limitation in a competitive context determines complex plant responses to experimental resource additions. *Ecology*, 94(11), 2505–2517. <https://doi.org/10.1890/12-1548.1>
- Fay, P. A., Prober, S. M., Harpole, W. S., Knops, J. M. H., Bakker, J. D., Borer, E. T., Lind, E. M., MacDougall, A. S., Seabloom, E. W., Wragg, P. D., Adler, P. B., Blumenthal, D. M., Buckley, Y. M., Chu, C., Cleland, E. E., Collins, S. L., Davies, K. F., Du, G., Feng, X., ... Yang, L. H. (2015). Grassland productivity limited by multiple nutrients. *Nature Plants*, 1(7), 15080. <https://doi.org/10.1038/nplants.2015.80>
- Ghannoum, O., Evans, J. R., & von Caemmerer, S. (2011). Chapter 8 nitrogen and water use efficiency of C4 plants. In A. S. Raghavendra & R. F. Sage (Eds.), *C4 photosynthesis and related CO₂ concentrating mechanisms* (pp. 129–146). Springer. https://doi.org/10.1007/978-90-481-9407-0_8
- Griffin-Nolan, R. J., Blumenthal, D. M., Collins, S. L., Farkas, T. E., Hoffman, A. M., Mueller, K. E., Ocheltree, T. W., Smith, M. D., Whitney, K. D., & Knapp, A. K. (2019). Shifts in plant functional composition following long-term drought in grasslands. *Journal of Ecology*, 107(5), 2133–2148. <https://doi.org/10.1111/1365-2745.13252>
- Gruner, D. S., Bracken, M. E. S., Berger, S. A., Eriksson, B. K., Gamfeldt, L., Matthiessen, B., Moorthi, S., Sommer, U., & Hillebrand, H. (2017). Effects of experimental warming on biodiversity depend on ecosystem type and local species composition. *Oikos*, 126(1), 8–17. <https://doi.org/10.1111/oik.03688>

- Hungate, B. A., Stiling, P. D., Dijkstra, P., Johnson, D. W., Ketterer, M. E., Hymus, G. J., Hinkle, C. R., & Drake, B. G. (2004). CO₂ elicits long-term decline in nitrogen fixation. *Science*, 304(5675), 1291.
- Isbell, F., Reich, P. B., Tilman, D., Hobbie, S. E., Polasky, S., & Binder, S. (2013). Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proceedings of the National Academy of Sciences of the United States of America*, 110(29), 11911–11916. <https://doi.org/10.1073/pnas.1310880110>
- Kimmel, K., Furey, G. N., Hobbie, S. E., Isbell, F., Tilman, D., & Reich, P. B. (2020). Diversity-dependent soil acidification under nitrogen enrichment constrains biomass productivity. *Global Change Biology*, 26, 6594–6603.
- Kirwan, L., Connolly, J., Finn, J. A., Brophy, C., Lüscher, A., Nyfeler, D., & Sebastià, M.-T. (2009). Diversity–interaction modeling: Estimating contributions of species identities and interactions to ecosystem function. *Ecology*, 90(8), 2032–2038. <https://doi.org/10.1890/08-1684.1>
- Knapp, A. K. (1993). Gas exchange dynamics in C3 and C4 grasses: Consequence of differences in stomatal conductance. *Ecology*, 74(1), 113–123. <https://doi.org/10.2307/1939506>
- Komatsu, K. J., Avolio, M. L., Lemoine, N. P., Isbell, F., Grman, E., Houseman, G. R., Koerner, S. E., Johnson, D. S., Wilcox, K. R., Alatalo, J. M., Anderson, J. P., Aerts, R., Baer, S. G., Baldwin, A. H., Bates, J., Beierkuhnlein, C., Belote, R. T., Blair, J., Bloor, J. M. G., ... Zhang, Y. (2019). Global change effects on plant communities are magnified by time and the number of global change factors imposed. *Proceedings of the National Academy of Sciences of the United States of America*, 116(36), 17867–17873. <https://doi.org/10.1073/pnas.1819027116>
- Ladouceur, E., Blowes, S. A., Chase, J. M., Clark, A. T., Garbowski, M., Alberti, J., Arnillas, C. A., Bakker, J. D., Barrio, I. C., Bharath, S., Borer, E. T., Brudvig, L. A., Cadotte, M. W., Chen, Q., Collins, S. L., Dickman, C. R., Donohue, I., Du, G., Ebeling, A., ... Harpole, W. S. (2022). Linking changes in species composition and biomass in a globally distributed grassland experiment. *Ecology Letters*, 25, 2699–2712. <https://doi.org/10.1111/ele.14126>
- Lawes, J. B., & Gilbert, J. H. (1880). Agricultural, botanical, and chemical results of experiments on the mixed herbage of permanent meadow, conducted for more than twenty years in succession on the same land—Part I. *Philosophical Transactions of the Royal Society of London*, 171, 289–416.
- Lee, T. D., Reich, P. B., & Tjoelker, M. G. (2003). Legume presence increases photosynthesis and N concentrations of co-occurring non-fixers but does not modulate their responsiveness to carbon dioxide enrichment. *Oecologia*, 137(1), 22–31. <https://doi.org/10.1007/s00442-003-1309-1>
- Lee, T. D., Tjoelker, M. G., Reich, P. B., & Russelle, M. P. (2003). Contrasting growth response of an N₂-fixing and non-fixing forb to elevated CO₂: Dependence on soil N supply. *Plant and Soil*, 255(2), 475–486. <https://doi.org/10.1023/A:1026072130269>
- Loreau, M. (2010). *From populations to ecosystems: Theoretical foundations for a new ecological synthesis*. Princeton University Press. <https://press.princeton.edu/books/paperback/9780691122700/from-populations-to-ecosystems>
- Mas, E., Cochard, H., Deluigi, J., Didion-Gency, M., Martin-StPaul, N., Morcillo, L., Valladares, F., Vilagrosa, A., & Grossiord, C. (2024). Interactions between beech and oak seedlings can modify the effects of hotter droughts and the onset of hydraulic failure. *New Phytologist*, 241(3), 1021–1034. <https://doi.org/10.1111/nph.19358>
- Mohanbabu, N., Isbell, F., Hobbie, S. E., & Reich, P. B. (2024). Data and code for “Species interactions amplify functional group responses to elevated CO₂ and N enrichment in a 24-year grassland experiment” [Dataset]. *Dryad*. <https://doi.org/10.5061/dryad.9zw3r22q5>
- Pearcy, R. W., & Ehleringer, J. (1984). Comparative ecophysiology of C3 and C4 plants. *Plant, Cell & Environment*, 7(1), 1–13. <https://doi.org/10.1111/j.1365-3040.1984.tb01194.x>
- Pennings, S. C., Clark, C. M., Cleland, E. E., Collins, S. L., Gough, L., Gross, K. L., Milchunas, D. G., & Suding, K. N. (2005). Do individual plant species show predictable responses to nitrogen addition across multiple experiments? *Oikos*, 110(3), 547–555. <https://doi.org/10.1111/j.0030-1299.2005.13792.x>
- Pinheiro, J., Bates, D., & R Core Team. (2023). *nlme: Linear and nonlinear mixed effects models*. <https://CRAN.R-project.org/package=nlme>
- Pirhofer-Walzl, K., Rasmussen, J., Høgh-Jensen, H., Eriksen, J., Sørensen, K., & Rasmussen, J. (2012). Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant and Soil*, 350(1–2), 71–84. <https://doi.org/10.1007/s11104-011-0882-z>
- Polley, H. W., Johnson, H. B., & Derner, J. D. (2003). Increasing CO₂ from subambient to superambient concentrations alters species composition and increases above-ground biomass in a C3/C4 grassland. *New Phytologist*, 160(2), 319–327. <https://doi.org/10.1046/j.1469-8137.2003.00897.x>
- Porter, H. O., Roberts, D. C., Tignor, M., Poloczanska, E. S., Mintenbeck, K., Alegria, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., & Rama, B. (2022). IPCC, 2022: Climate change 2022: Impacts, adaptation, and vulnerability. In V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, & M. Huang (Eds.), *Contribution of working group II to the sixth assessment report of the intergovernmental panel on climate change*. Cambridge University Press.
- R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Reich, P. B. (2009). Elevated CO₂ reduces losses of plant diversity caused by nitrogen deposition. *Science*, 326(5958), 1399–1402. <https://doi.org/10.1126/science.1178820>
- Reich, P. B., Hobbie, S. E., & Lee, T. D. (2014). Plant growth enhancement by elevated CO₂ eliminated by joint water and nitrogen limitation. *Nature Geoscience*, 7(12), 920–924. <https://doi.org/10.1038/ngeo2284>
- Reich, P. B., Hobbie, S. E., Lee, T. D., & Pastore, M. A. (2018). Unexpected reversal of C3 versus C4 grass response to elevated CO₂ during a 20-year field experiment. *Science*, 360(6386), 317–320.
- Reich, P. B., Hobbie, S. E., Lee, T. D., Rich, R., Pastore, M. A., & Worm, K. (2020). Synergistic effects of four climate change drivers on terrestrial carbon cycling. *Nature Geoscience*, 13(12), 787–793. <https://doi.org/10.1038/s41561-020-00657-1>
- Reich, P. B., Knops, J., Tilman, D., Craine, J., Ellsworth, D., Tjoelker, M., Lee, T., Wedin, D., Naeem, S., Bahaeddin, D., Hendrey, G., Jose, S., Wragge, K., Goth, J., & Bengtson, W. (2001). Plant diversity enhances ecosystem responses to elevated CO₂ and nitrogen deposition. *Nature*, 410(6830), 809–812. <https://doi.org/10.1038/35071062>
- Reich, P. B., Tilman, D., Craine, J., Ellsworth, D., Tjoelker, M. G., Knops, J., Wedin, D., Naeem, S., Bahaeddin, D., Goth, J., Bengtson, W., & Lee, T. D. (2001). Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO₂ and N availability regimes? A field test with 16 grassland species. *New Phytologist*, 150(2), 435–448. <https://doi.org/10.1046/j.1469-8137.2001.00114.x>
- Reich, P. B., Tilman, D., Naeem, S., Ellsworth, D. S., Knops, J., Craine, J., Wedin, D., & Trost, J. (2004). Species and functional group diversity independently influence biomass accumulation and its response to CO₂ and N. *Proceedings of the National Academy of Sciences of the United States of America*, 101(27), 10101–10106. <https://doi.org/10.1073/pnas.0306602101>
- Ren, F., Song, W., Chen, L., Mi, Z., Zhang, Z., Zhu, W., Zhou, H., Cao, G., & He, J.-S. (2016). Phosphorus does not alleviate the negative effect of nitrogen enrichment on legume performance in an alpine grassland. *Journal of Plant Ecology*, 10, rtw089. <https://doi.org/10.1093/jpe/rtw089>
- Smith, M. D., Knapp, A. K., & Collins, S. L. (2009). A framework for assessing ecosystem dynamics in response to chronic resource alterations

- induced by global change. *Ecology*, 90(12), 3279–3289. <https://doi.org/10.1890/08-1815.1>
- Spehn, E. M., Scherer-Lorenzen, M., Schmid, B., Hector, A., Caldeira, M. C., Dimitrakopoulos, P. G., Finn, J. A., Jumpponen, A., Donovan, G. O., Pereira, J. S., Schulze, E.-D., Troumbis, A. Y., & Körner, C. (2002). The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. *Oikos*, 98(2), 205–218.
- Suding, K. N., Collins, S. L., Gough, L., Clark, C., Cleland, E. E., Gross, K. L., Milchunas, D. G., & Pennings, S. (2005). Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences of the United States of America*, 102(12), 4387–4392. <https://doi.org/10.1073/pnas.0408648102>
- Tian, D., & Niu, S. (2015). A global analysis of soil acidification caused by nitrogen addition. *Environmental Research Letters*, 10(2), 024019. <https://doi.org/10.1088/1748-9326/10/2/024019>
- Tilman, D. (1982). *Resource competition and community structure*. Princeton University Press.
- Tognetti, P. M., Prober, S. M., Báez, S., Chaneton, E. J., Firn, J., Risch, A. C., Schuetz, M., Simonsen, A. K., Yahdjian, L., Borer, E. T., Seabloom, E. W., Arnillas, C. A., Bakker, J. D., Brown, C. S., Cadotte, M. W., Caldeira, M. C., Daleo, P., Dwyer, J. M., Fay, P. A., ... Sankaran, M. (2021). Negative effects of nitrogen override positive effects of phosphorus on grassland legumes worldwide. *Proceedings of the National Academy of Sciences of the United States of America*, 118(28), e2023718118. <https://doi.org/10.1073/pnas.2023718118>
- van Groenigen, K.-J., Six, J., Hungate, B. A., de Graaff, M.-A., van Breemen, N., & van Kessel, C. (2006). Element interactions limit soil carbon storage. *Proceedings of the National Academy of Sciences of the United States of America*, 103(17), 6571–6574. <https://doi.org/10.1073/pnas.0509038103>
- Vandermeer, J. (1981). The interference production principle: An ecological theory for agriculture on JSTOR. *Bioscience*, 31(5), 361–364.
- Vázquez, E., Schleuss, P.-M., Borer, E. T., Bugalho, M. N., Caldeira, M. C., Eisenhauer, N., Eskelinen, A., Fay, P. A., Haider, S., Jentsch, A., Kirkman, K. P., McCulley, R. L., Peri, P. L., Price, J., Richards, A. E., Risch, A. C., Roscher, C., Schütz, M., Seabloom, E. W., ... Spohn, M. (2022). Nitrogen but not phosphorus addition affects symbiotic N₂ fixation by legumes in natural and semi-natural grasslands located on four continents. *Plant and Soil*, 478(1), 689–707. <https://doi.org/10.1007/s11104-022-05498-y>
- Ward, S. J. E., Midgley, G. Y. F., Jones, M. H., & Curtis, P. S. (1999). Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO₂ concentration: A meta-analytic test of current theories and perceptions. *Global Change Biology*, 5(6), 723–741. <https://doi.org/10.1046/j.1365-2486.1999.00265.x>
- Warren, J. M., Jensen, A. M., Medlyn, B. E., Norby, R. J., & Tissue, D. T. (2015). Carbon dioxide stimulation of photosynthesis in *Liquidambar styraciflua* is not sustained during a 12-year field experiment. *AoB Plants*, 7, plu074. <https://doi.org/10.1093/aobpla/plu074>
- Wedin, D. A., & Tilman, D. (1996). Influence of nitrogen loading and species composition on the carbon balance of grasslands. *Science*, 274, 1720–1723.
- West, J. B., HilleRisLambers, J., Lee, T. D., Hobbie, S. E., & Reich, P. B. (2005). Legume species identity and soil nitrogen supply determine symbiotic nitrogen-fixation responses to elevated atmospheric [CO₂]. *New Phytologist*, 167(2), 523–530. <https://doi.org/10.1111/j.1469-8137.2005.01444.x>
- Wright, A. J., Wardle, D. A., Callaway, R., & Gaxiola, A. (2017). The overlooked role of facilitation in biodiversity experiments. *Trends in Ecology & Evolution*, 32(5), 383–390. <https://doi.org/10.1016/j.tree.2017.02.011>
- Yue, K., Peng, Y., Fornara, D. A., Van Meerbeek, K., Vesterdal, L., Yang, W., Peng, C., Tan, B., Zhou, W., Xu, Z., Ni, X., Zhang, L., Wu, F., & Svenning, J.-C. (2019). Responses of nitrogen concentrations and pools to multiple environmental change drivers: A meta-analysis across terrestrial ecosystems. *Global Ecology and Biogeography*, 28(5), 690–724. <https://doi.org/10.1111/geb.12884>
- Zavaleta, E. S., Shaw, M. R., Chiariello, N. R., Mooney, H. A., & Field, C. B. (2003). Additive effects of simulated climate changes, elevated CO₂, and nitrogen deposition on grassland diversity. *Proceedings of the National Academy of Sciences of the United States of America*, 100(13), 7650–7654. <https://doi.org/10.1073/pnas.0932734100>
- Zeng, D.-H., Li, L.-J., Fahey, T. J., Yu, Z.-Y., Fan, Z.-P., & Chen, F.-S. (2010). Effects of nitrogen addition on vegetation and ecosystem carbon in a semi-arid grassland. *Biogeochemistry*, 98(1/3), 185–193.

SUPPORTING INFORMATION

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

How to cite this article: Mohanbabu, N., Isbell, F., Hobbie, S. E., & Reich, P. B. (2024). Species interactions amplify functional group responses to elevated CO₂ and N enrichment in a 24-year grassland experiment. *Global Change Biology*, 30, e17476. <https://doi.org/10.1111/gcb.17476>