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Flowering in grassland predicted by CO_2 and resource effects on species aboveground biomass

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Abstract

Continuing enrichment of atmospheric CO_2 may change plant community composition, in part by altering the availability of other limiting resources including soil water, nutrients, or light. The combined effects of CO_2 enrichment and altered resource availability on species flowering remain poorly understood. We quantified flowering culm and ramet production and biomass allocation to flowering culms/ramets for 10 years in C_4-dominated grassland communities on contrasting soils along a CO_2 concentration gradient spanning pre-industrial to expected mid-21st century levels (250–500 μl/L). CO_2 enrichment explained up to 77% of the variation in flowering culm count across soils for three of the five species, and was correlated with flowering culm count on at least one soil for four of five species. In contrast, allocation to flowering culms was only weakly correlated with CO_2 enrichment for two species. Flowering culm counts were strongly correlated with species aboveground biomass (AGB; $R^2 = .34$–$.74$), a measure of species abundance. CO_2 enrichment also increased soil moisture and decreased light levels within the canopy but did not affect soil inorganic nitrogen availability. Structural equation models fit across the soils suggested species-specific controls on flowering in two general forms: (1) CO_2 effects on flowering culm count mediated by canopy light level and relative species AGB (species AGB/total AGB) or by soil moisture effects on flowering culm count; (2) effects of canopy light level or soil inorganic nitrogen on flowering and/or relative species AGB, but with no significant CO_2 effect. Understanding the heterogeneity in species responses to CO_2 enrichment in plant communities across soils in edaphically variable landscapes is critical to predict CO_2 effects on flowering and other plant fitness components, and species potential to adapt to future environmental changes.

KEYWORDS
allocation, biodiversity, greenhouse gases, life history, Poaceae, primary productivity, soil texture, tallgrass prairie

1 | INTRODUCTION

The concentration of atmospheric CO_2 continues to increase and is expected to exceed 500 μl/L by 2050 and, without mitigation, approach 1,000 μl/L by 2100 (Collins et al., 2013). Atmospheric CO_2 enrichment usually increases the productivity of plant communities (Ainsworth & Long, 2005), often because of sizeable contributions from species for which abundance increases with CO_2 enrichment (Fay, Newingham, et al., 2015; Gornish & Tylianakis, 2013; Polley, Jin, & Fay, 2012b). The response of flowering to CO_2 enrichment has received less attention despite its importance for vegetation dynamics.

CO_2-caused variation in flowering may arise through two main pathways. CO_2 enrichment may increase flowering by increasing plant size alone if inflorescences account for a fixed proportion of plant biomass, such that larger or more abundant plants of a species produce more inflorescences in a given area than smaller or less abundant ones. Alternatively, CO_2 enrichment may increase inflorescence production by changing the allocation of resources between vegetative and reproductive growth. Changes in allocation to flowering suggest that flowering differs in type or degree of resource limitation compared to vegetative growth. For example, flowering and seed production are generally considered more expensive in terms of C and nutrients than vegetative growth (Bazzaz, Chiariello, Coley, & Pitelka, 1987; Harper, 1977; McGinley & Charnov, 1988). Thus, CO_2 enrichment could increase allocation to flowering if flowering is more C-limited than vegetative growth. Conversely, CO_2 enrichment could reduce allocation to flowering if flowering is more limited by soil moisture, nutrients, or light availability and CO_2 enrichment decreases their availability (Luo et al., 2004).

Soil properties are likely to mediate the effects of CO_2 enrichment on flowering. Soil parent material and texture affect soil water holding capacity and soil nutrient availability, and therefore the magnitude of water and nutrient limitation (Augusto, Achat, Jonard, Vidal, & Ringeval, 2017; Maestre & Reynolds, 2007). For example, fine-textured clay soils often have greater water holding capacity and organic matter and nutrient contents than coarse-textured sandy soils (Brady & Weil, 2002). However, greater retention of water in clay soils has been shown to limit elevated CO_2-induced increases in community annual net primary productivity (ANPP) compared to sandy soils (Fay, Jin, et al., 2012). Soil heterogeneity across landscapes can thus lead to considerable spatial heterogeneity in flowering responses to CO_2 enrichment.

Meta-analyses have suggested general patterns in the effects of CO_2 enrichment on flowering and reproduction. For example, for a sample of over 160 native plant species CO_2 enrichment marginally decreased allocation to flowering (Wang, Taub, & Jablonski, 2015) because CO_2 enrichment increased reproductive biomass only half as much as vegetative biomass. In contrast, across nearly 520 crop species (Wang et al., 2015), reproductive allocation increased almost 14%, at elevated CO_2 and was accompanied by up to 28% higher fruit and seed production, compared to only 4% higher for native

species (Jablonski, Wang, & Curtis, 2002). The effects of nutrient or water availability on the relationship between CO_2 enrichment and flowering remain less clear. Across 53 species, nutrient stress did not alter the effects of CO_2 enrichment on reproductive allocation (Wang et al., 2015), but the outcome in specific cases likely depends on the degree of nutrient limitation. For example, in a N-limited grassland, inflorescence production was reduced by CO_2 enrichment (HilleRisLambers, Harpole, Schnitzer, Tilman, & Reich, 2009). Drought stress interacted with CO_2 enrichment to increase reproductive biomass more than vegetative biomass, thereby increasing reproductive allocation (Wang et al., 2015).

The combined effects of CO_2 enrichment and resource availability on flowering directly affect subsequent components of plant fitness including seed numbers and size, germination rates, and seedling success. For example, CO_2 enrichment increased seed dispersal by increasing inflorescence numbers per m^2 and seed numbers per inflorescence, which translated into increased seedling recruitment (Edwards, Clark, & Newton, 2001). However, the effects of CO_2 enrichment on seed mass can increase in some species while decreasing in others in the same community (Hovenden et al., 2007). HilleRisLambers et al. (2009) concluded that inflorescence production was the main driver of CO_2 effects on allocation to seed production in grassland. The genetic variability contributed by flowering directly relates to the potential for evolutionary change and species long-term persistence in a changing environment (Becklin et al., 2016; Franks, Weber, & Aitken, 2014).

In this study, our objective was to examine whether CO_2 enrichment altered the production and the allocation of biomass to flowering, here defined as the production of inflorescences, in perennial grassland plant communities. We also tested whether CO_2 effects on flowering and allocation varied with co-occurring changes in soil moisture, soil nutrients, and light levels within the canopy across a range of fine- to coarse-textured soils. To our knowledge, the combined effects of CO_2 enrichment and light levels within canopies on allocation to flowering have not been examined. We measured flowering culm or ramet production and allocation in experimentally constructed plant communities composed of native perennial prairie grasses (C_4) and forbs in a 10-year CO_2 enrichment experiment with CO_2 concentrations spanning pre-industrial to anticipated mid-21st century values (Fay et al., 2009; Polley, Johnson, Fay, & Sanabria, 2008). The communities were established on intact monoliths of clay, silty clay, and sandy loam soils representing lowland, upland, and riparian soils, respectively, in the Blackland Prairie region of central Texas, USA.

We previously showed soil-mediated effects of CO_2 enrichment on community ANPP (Fay, Newingham, et al., 2015; Polley, Jin, & Fay, 2012a; Polley et al., 2012b). Atmospheric CO_2 enrichment strongly increased ANPP on a silty clay soil because of increased biomass of a productive mesic tallgrass species and the decline of a drought-tolerant mid-grass species, the biomass of which increased at lower CO_2 concentrations. Aboveground NPP increased with CO_2 enrichment on a sandy loam soil where lower transpiration translated into increased soil water content. We predicted that (1) the

number of flowering culms or ramets per unit area would vary with CO_2 enrichment depending on how CO_2 enrichment and CO_2-associated changes in canopy light level, soil moisture, or soil N combined to affect species aboveground biomass (AGB), a measure of species abundance, and (2) changes in allocation to flowering culms or ramets with CO_2 enrichment would be found when CO_2 enrichment and resources combined to affect both species AGB and numbers of flower culms/ramets.

2 | MATERIALS AND METHODS

2.1 | Study site and experimental design

2.1.1 | Site description

The study was conducted in the Lysimeter CO_2 Gradient (LYCOG) facility, located at Temple, TX, USA (31°05′ N, 97°20′ W) in the southern US Central Plains. The climate at this site is subtropical, falling in the transition between humid and sub-humid zones. Mean annual precipitation is 917 mm (1981–2010), with growing season wet periods in May–June and September–October, and a pronounced July–August dry period. Temperatures range from a July–August mean maximum of 35°C to a December mean minimum of 2.9°C. The mean frost free period is ~250 days, from mid-March to late November.

2.1.2 | CO_2 chambers

The CO_2 gradient experiment was conducted in two outdoor linear chambers each composed of ten 5-m long × 1.2-m wide sections (Fay, Reichmann, Aspinwall, Khasanova, & Polley, 2015). Each 5-m section was enclosed with clear polyethylene (0.006″/0.15 mm thickness). This film transmits >90% of incident light with minimal effects on spectral quality, and is similar to polyethylene films used in other global change experiments (e.g., Dermody, Weltzin, Engel, Allen, and Norby, 2007). The chambers contained intact soil monoliths (1 m² area × 1.5 m deep) from three contrasting soil series common to the Texas Blackland Prairie Region: a silty clay Mollisol (Austin series, $n = 32$), a sandy loam Alfisol (Bastsil series, $n = 16$), and a clay Vertisol (Houston Black series, $n = 32$). Monoliths were excavated and encased in steel boxes in 2002 and used for the duration of the experiment (Fay et al., 2009; Polley et al., 2008). Within each 5-m section, two of the three soil types were paired in duplicate in random order, with the sandy loam included in alternating sections.

2.1.3 | Grassland communities

Experimental communities were planted in the monoliths in spring 2003. Seedlings of four C_4 grasses, two C_3 forbs, and one herbaceous legume were planted in a Latin Square design, detailed in Polley et al. (2008). All were perennials and native to Texas Blackland Prairie, the original natural vegetation at this location. Other species appearing in later years were regularly removed by hand or selective

glyphosate application. Our focus here is on the most abundant of the species over the 10-year experiment, specifically the four C_4 grasses *Sorghastrum nutans* (L.) Nash, *Bouteloua curtipendula* (Michx.) Torr., *Schizachyrium scoparium* (Michx.) Nash, *Tridens albescens* (Vasey) Wooton & Standl், and one C_3 forb, *Solidago canadensis* L., hereafter referred to by genus. All are widespread and common in the Central Plains grasslands of North America. *Sorghastrum*, *Solidago*, and *Tridens* are typically found in more mesic locations with deeper soils, while *Bouteloua* and *Schizachyrium* more often occupy drier locations. The two remaining members of the experimental communities, the C_3 forb *Salvia azurea* Michx. ex Lam. and the herbaceous legume *Desmanthus illinoensis* (Michx.) MacMill. ex B.L. Rob. & Fernald, were too rare across the three soils to assess soil-specific differences in flowering. In 2007, 20 monoliths (8 silty clay, 12 clay) were replanted to switchgrass (*Panicum virgatum*) to improve CO_2 control by increasing photosynthetic sink strength (Fay, Polley, Jin, & Aspinwall, 2012). This left 60 monoliths (silty clay $n = 24$, sandy loam $n = 16$, and heavy clay $n = 20$) in the grassland experiment.

2.1.4 | CO_2 treatments

A daytime linear CO_2 gradient of 500–250 μl/L was maintained during April–October of each growing season from 2006 to 2015. The gradient was initiated by introducing air enriched to 500 μl/L CO_2 into the first linear chamber. Fans advected this air through successive sections, and photosynthesis by the enclosed vegetation progressively depleted the air of CO_2. The air flow rate was controlled so that air exited the elevated chamber at 380 μl/L CO_2. Similarly, ambient air was introduced to the second linear chamber and exited at 250 μl/L. The plant communities were exposed to ambient conditions during winter. Atmospheric CO_2 measurement and control procedures are further detailed in Polley et al. (2008) and Fay, Reichmann, et al. (2015). Air temperature in the chambers was controlled to match outside ambient temperature (Fay et al., 2009). Each monolith was watered twice a week in events summing to the average growing season rainfall amount for this locale when the treatments were initiated (1971–2000: 560 mm), which is near the current (1981–2010) value of 578 mm. The seasonal pattern of irrigation was varied among years to introduce realistic variation in spring and summer rainfall, by shifting between wetter springs/drier summers, the typical ambient pattern, and the opposite on 1- or 2-year cycles.

2.2 | Measurement of plant responses

2.2.1 | Flowering rates

Grass flowering culms were counted in each 1 m² monolith at peak flowering for each species, typically July for *Bouteloua* and *Tridens*, and September for *Sorghastrum*, and *Schizachyrium*. For *Solidago*, we counted the number of flowering and non-flowering ramets in September or October. Hereafter, we use "flowering culms" to refer collectively to grass and *Solidago* counts.

2.2.2 | Biomass components

Species AGB was measured each November to estimate species abundance. All AGB was clipped by species at 5 cm height, dried for 72 hr at 60°C, and weighed. Species AGB was summed to estimate total community AGB (total AGB). Mass per flowering culm for the grasses was measured in 2015 from the dry weights of 10–15 mature flowering culms per species from each monolith. In preliminary analysis, mass per flowering culm did not vary with soils or CO_2 for any species, so yearly flowering culm biomass was estimated as the product of the yearly flowering culm counts and the 2015 mean mass per flowering culm, averaged across soils and CO_2. Allocation to flowering culms was then estimated for the grasses as the ratio of flowering biomass to species AGB, and for *Solidago* as the ratio of the proportion of ramets flowering to species AGB.

2.3 | Measurement of resource availabilities

2.3.1 | Soil moisture

Volumetric soil water content over 0–40 cm depth (SWC_{40}) was measured weekly each year, except biweekly in 2006, with a calibrated neutron attenuation probe (503DR Hydroprobe, CPN International, Concord, CA, USA) at permanently installed access tubes.

2.3.2 | Plant-available soil nitrogen

Soil NO_3^- and NH_4^+ in soil solution was estimated in 2008, 2009, 2010, 2013, and 2015 using ion exchange resins (PRS® probes; Western Ag Innovations Inc., Saskatoon, SK, Canada). Probes were installed monthly during CO_2 treatment. Probes from monolith pairs within each section were pooled for colorimetric analysis of NO_3^--N and NH_4^+-N, which were summed to estimate total resin-available inorganic N (TIN) in μg per 10 cm^{-2}, the area of resin exposed to soil.

2.3.3 | Canopy light levels

Photosynthetic photon flux density at 10 cm above the soil surface ($PPFD_{10}$) in the plant canopy of each monolith was measured once each July using a ceptometer (SunScan®; Delta-T Devices Ltd., Burwell, Cambridge, UK). Each measurement was the average of two $PPFD_{10}$ readings with the ceptometer placed on each diagonal, made when the elevation angle of the sun was less than 60 degrees and ambient light levels exceeded 600 μmol m^{-2} s^{-1}. For this analysis, we further excluded readings with ambient light <1,000 μmol m^{-2} s^{-1}.

2.4 | Data analysis

2.4.1 | Data preparation

Flowering culm count, species AGB, $PPFD_{10}$, SWC_{40}, and TIN values more extreme than 1.5× the interquartile range were removed as outliers prior to analysis. This removed 4% of species AGB observations and 6.5% of flowering culms counts out of 2100 observations. Observations were omitted from further analysis in three other cases: (1) silty clay monoliths in two sections where one member of the pair was planted to switchgrass; (2) *Sorghastrum* in the first section of the enriched CO_2 chamber because it grew unexpectedly poorly there compared to the rest of the enriched chamber and (3) where a small number of flowering culms were counted but no AGB was later recorded because of low abundance. Yearly plant and soil responses were averaged by soil type within each 5-m section. For structural equation modeling (SEM) (see below), we further averaged responses across years.

2.4.2 | Statistical models

We ran linear mixed models in SAS/STAT® 13.1 (SAS Institute Inc., 2013) to test whether flowering culm count, species AGB, and allocation to flowering varied with CO_2 enrichment, and whether responses to CO_2 enrichment varied among soil series. First, an analysis of covariance model (Equation 1) was fit to flowering culm counts using a generalized linear mixed model procedure (Proc GLIMMIX) with Poisson errors and a log link. Equation (1) was fit to species AGB and reproductive allocation with a linear mixed model (Proc MIXED):

$$y_{ijkl} = \text{intercept} + \text{soil}_i + \alpha(CO_2) + \beta(CO_2 \times \text{soil}_i) + \text{species}_k \\ + \text{soil} \times \text{species}_{ik} + \gamma(CO_2 \times \text{species}_k) \\ + \delta(CO_2 \times \text{soil} \times \text{species}_{ik}) + e_{ijkl} \tag{1}$$

Monolith nested within soil type was fit as a random effect, and year was fit as a repeated effect with an autoregressive covariance structure. Interactions of year with soil, species, and CO_2 were not fit to improve convergence and because the objective was to identify general patterns across years.

Second, a reduced model (Equation 2) was fit to flowering culm count, species AGB, and reproductive allocation for each species individually:

$$y_{ijk} = \text{intercept} + \text{soil}_i + \text{monolith}_j(\text{soil}_i) + \alpha(CO_2) + \beta(CO_2 \times \text{soil}_i) + e_{ijk} \tag{2}$$

Equation (2) was also fit to SWC_{40}, total AGB, TIN, and $PPFD_{10}$ to evaluate CO_2 and soil effects on other potential drivers of flowering culm count. When these analyses indicated that flowering culm count or biomass variables were related to CO_2 or species AGB, we fit linear functions using OriginPro 9.3. For *Sorghastrum* flowering culms–CO_2 relationships, we fit exponential functions when supported by improvements in Akaike's Information Criteria, Akaike Weight Ratios, and Bayesian Information Criteria (Table S2).

We developed a SEM of flowering culm counts to account for the individual and joint effects of CO_2 enrichment, soil moisture, canopy light levels, and soil inorganic nitrogen on species abundance and flowering culm counts. For the SEMs, we made two substitutions: (1) relative species AGB (species AGB/total AGB) in place of absolute species AGB to adjust for covariance among species AGB,

PPFD$_{10}$, and total AGB, and (2) the log response ratio of SWC$_{40}$ (lrrSWC$_{40}$) centered on the mean SWC$_{40}$ for each soil type, to factor out among-soil differences in soil moisture. SEMs were fit separately to each species to evaluate the species-specific contributions of these inter-correlated variables to flowering culm production. For each species, the SEM was fit across soils to test for general patterns emerging across soil types.

Specifically, the SEM contained (1) direct CO$_2$ effects on relative species AGB and flowering culm count, hypothesized to represent physiological effects of CO$_2$ on species arising from increased carboxylation and water use efficiencies; (2) direct CO$_2$ effects on lrrSWC$_{40}$ and PPFD$_{10}$, hypothesized to represent community scale effects of CO$_2$ on soil moisture and canopy light level; (3) effects of PPFD$_{10}$, lrrSWC$_{40}$, and TIN on relative abundance and flowering culm count, hypothesized to represent effects of light, soil moisture, and nitrogen availability on relative species AGB and flowering. SEM modeling was conducted using Proc CALIS. Path coefficients were standardized by the variance ratio of the two variables forming the path. Non-significant paths were retained in the final models but not drawn in the figure for clarity. Model fit for each species (Table 1) was evaluated using chi-square and indices that correct for sample size (CFI), that measure deviations of residuals from a hypothesized covariance model (SRMR), and that favor higher parsimony (RMSEA, NNFI) following Hooper, Coughlan, and Mullen (2008).

3 | RESULTS

3.1 | Allocation to flowering culms and CO$_2$ enrichment

Allocation to flowering culms of some species responded to CO$_2$ enrichment (CO$_2$ × species, $p < .0001$, Table S1), but not as consistently as expected. Across soils, allocation to flowering culms was a decreasing function of CO$_2$ enrichment in *Bouteloua*, and an increasing function of CO$_2$ enrichment in *Schizachyrium* ($p < .01$, data not shown). However, CO$_2$ enrichment explained <10% of the total variation in allocation for these species. Allocation was not correlated with CO$_2$ enrichment for *Solidago, Sorghastrum, or Tridens* ($p = .06–.92$, Table S1).

Atmospheric CO$_2$ enrichment explained up to 77% of the variation in flowering culm count across soils for three of the five species and was correlated with flowering culm count on at least one soil for four of five species. Flowering culm count increased with CO$_2$

enrichment across soils for *Sorghastrum* and *Solidago* ($R^2 = .24–.58$, $p < .003$, Tables S1 and S2; Figure 1a,b), and on the silty clay soil for *Schizachyrium* ($R^2 = .56$, $p < .005$, Figure 1d). In *Sorghastrum*, flowering culm counts increased rapidly above ~400 µl/L CO$_2$ on silty clay and clay soils ($R^2 = .58–.77$, $p < .04$, Table S2, Figure 1a,b), indicating a larger response to superambient than to subambient CO$_2$ changes. In contrast, in *Bouteloua* there was a weak linear decrease ($R^2 = .03$, $p = .0004$) in flowering culm count with CO$_2$ across soils (Tables S1 and S2; Figure 1c), which was most pronounced on the sandy loam soil ($R^2 = .40$, $p = .05$; Table S2, Figure 1c). *Tridens* flowering culm count was unrelated to CO$_2$ enrichment ($p > .37$, Table S1; Figure 1e), and *Tridens* was not present on the sandy loam.

3.2 | Predictors of rates of flowering

Flowering culm count responses to CO$_2$ enrichment generally reflected the responses of species AGB to CO$_2$ enrichment. Species AGB increased with CO$_2$ enrichment exponentially across soils for *Sorghastrum*, linearly across soils for *Solidago*, and linearly on silty clay for *Tridens* ($R^2 = .21–.58$, $p < .03$; Table S2, Figure S1). In contrast, species AGB decreased with CO$_2$ across soils in *Bouteloua* although with considerable variability ($R^2 = .03$, $p < .05$; Table S2, Figure S1). As expected, flowering culm counts in all species increased linearly with species AGB across soils ($R^2 = .34–.74$, $p < .0001$; Table S2; Figure 2), and specifically on silty clay and clay soils for *Solidago* and *Schizachyrium* ($R^2 = .31–.86$, Figure 2b,d; Table S2).

CO$_2$ enrichment altered resource availability on some or all soils. SWC$_{40}$ on the sandy loam averaged ~twofold lower and increased up to threefold more with CO$_2$ enrichment ($R^2 = .52$, $p = .03$, Figure 3a) compared to the clay and silty clay soil ($p < .0001$, Figure 3a). PPFD$_{10}$ decreased across soils with CO$_2$ enrichment ($p < .0001$, Figure 3b). 70% of the variation in PPFD$_{10}$ was accounted for by variation in total AGB (Figure 3c, inset), which across soils nearly doubled from subambient to enriched CO$_2$ ($p < .0001$, Figure 3b). Soil inorganic N availability (TIN) was unrelated to CO$_2$ enrichment ($p = .063$) and did not differ among soils ($p = .50$, Figure 3c).

Structural equation models (Figure 4) achieved good fit for all species based on indices with sensitivity to sample size, parsimony, and residuals ($\chi^2 \leq .45$, $p \leq .50$; RMSEA = 0; SRMR ≤ 0.03; CFI = 1.0, NNFI ≥ 1.18, Table 1). The SEMs identified no direct

TABLE 1 Structural equation model fit statistics for each species (Hooper et al., 2008)

Species	Index Significance threshold	χ^2 (*p*-value) $p > .05$	RMSEA <0.06	SRMR <0.05	CFI >0.95	NNFI >0.95
Sorghastrum nutans		.45 (.50)	0	0.03	1.0	1.18
Solidago canadensis		.19 (.66)	0	0.02	1.0	1.34
Bouteloua curtipendula		.0004 (.98)	0	0.0009	1.0	1.40
Schizachyrium scoparium		.06 (.80)	0	0.015	1.0	1.28
Tridens albescens		.33 (.57)	0	0.03	1.0	1.33

χ^2, chi-square statistic; RMSEA, root mean square error of approximation; SRMR, standardized root mean square residual; CFI, Bentler Comparative Fit Index; NNFI, non-normed fit index.
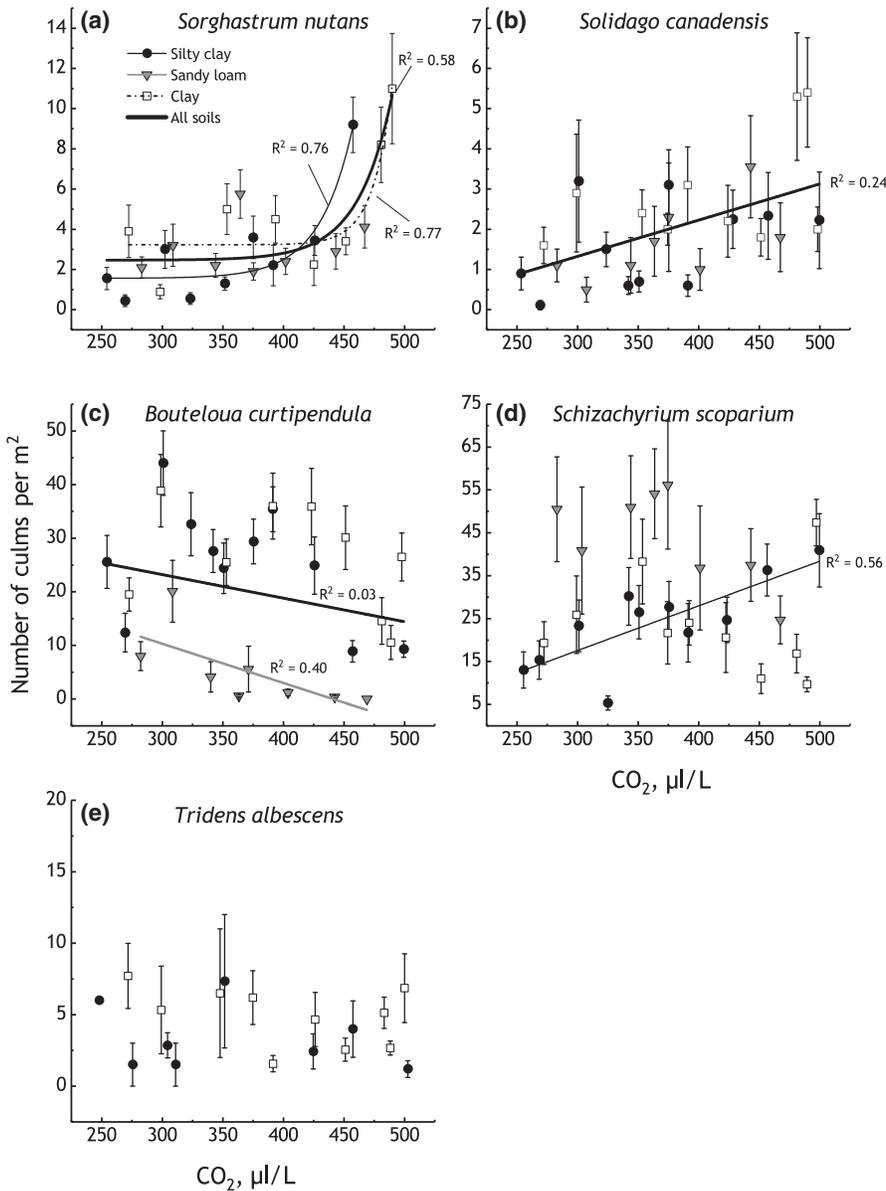
FIGURE 1 Flowering culm counts/m^2 in relation to CO_2 concentration on silty clay, sandy loam, and clay soils. Each datum represents the mean $\pm$ *SE* of 10 years of CO_2 treatment. Regression lines are plotted when there was significant fit for individual soil types or for all soils combined. Regression equations are presented in TableS2

effects of CO_2 enrichment on flowering culm count ($p = .14$–.96). Instead, SEMs identified significant total CO_2 effects on flowering culm count in *Sorghastrum* and *Solidago* ($p \leq .0001$, Table 2). CO_2 effects on count in *Solidago* were mediated by IrrSWC$_{40}$, and in *Sorghastrum* by the sequence PPFD$_{10}$-relative species AGB, augmented by a path connecting CO_2 and relative species AGB. These parallel paths in *Sorghastrum* combined to form the largest total CO_2 effect on inflorescence production among these species (0.637, $p < .0001$). For *Bouteloua*, *Schizachyrium*, and *Tridens*, SEMs indicated no CO_2 effects on flowering culm count (Table 2). In *Bouteloua*, CO_2 effects on count were absent despite a sequence of significant paths linking CO_2 to count because each path transmits only a fraction of the preceding effects, diminishing the initial CO_2 effect. *Schizachyrium* and *Tridens* lacked a sequence of paths from CO_2 to count.

PPFD$_{10}$ influenced flowering culm count in four of the five species, whether or not CO_2 affected PPFD$_{10}$. SEMs indicated

significant total PPFD$_{10}$ effects on flowering culm count in *Sorghastrum* (-0.333, $p = .048$) and *Solidago* (-0.345, $p = .033$) although significant direct or relative species AGB-mediated effects were not always resolved (Table 2). In contrast, for *Bouteloua* and *Tridens*, direct and relative species AGB-mediated effects of PPFD$_{10}$ were offsetting, resulting in no significant PPFD$_{10}$ effect. *Schizachyrium* lacked the direct and indirect paths by which CO_2 and PPFD$_{10}$ affected relative species AGB and flowering culm count in the other species. Instead, flowering culm counts were indirectly related to TIN, mediated by relative species AGB.

4 | DISCUSSION

We predicted that numbers of flowering culms would vary with CO_2 enrichment depending on how CO_2 and resource availability
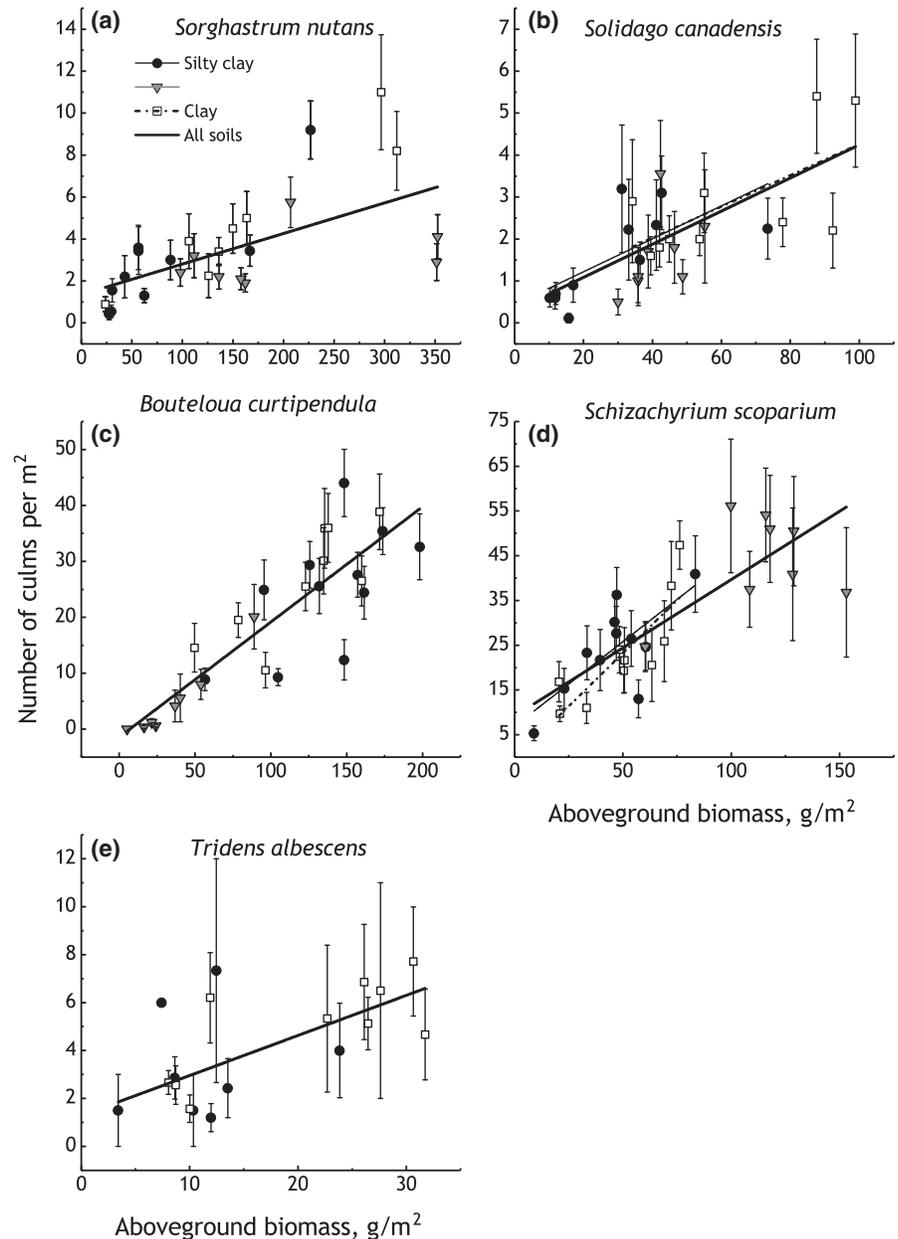
FIGURE 2 Flowering culm counts in relation to species aboveground biomass on silty clay, sandy loam, and clay soils. Each datum represents the mean $\pm$ SE of 10 years of CO_2 treatment. Regression lines are plotted when there was significant fit for individual soil types or for all soils combined. Regression equations are presented in TableS2

combined to affect species AGB, a measure of species abundance. SEM of flowering culm production partially supported this prediction for some species, based on results from 10 years of experimental exposure of grassland to a pre-industrial to mid-21st century range of CO_2 concentrations. We also predicted that changes in allocation to flowering culms/ramets with CO_2 enrichment would be found when CO_2 enrichment and resources combined to affect both species AGB and numbers of flowering culms/ramets. Our analyses did not support this prediction, providing at best weak evidence for CO_2 effects on allocation to flowering culms in two species for which SEMs did not include joint CO_2 or resource effects on flowering culm counts and relative species AGB. Furthermore, even in the species for which SEMs included joint CO_2 or resource effects on flowering culm counts and relative species AGB, we found no evidence for CO_2 effects on reproductive allocation.

4.1 | Prediction 1

Structural equation models resolved experimental CO_2 enrichment effects on flowering culm counts for two species, *Sorghastrum* and *Solidago*. In both cases, SEMs contained multiple pathways connecting CO_2 with flowering culm count, suggesting that CO_2 enrichment acted on flowering culm production through multiple mechanisms. CO_2 enrichment also increased aboveground net primary productivity to a greater degree in this experiment when multiple reinforcing paths were present (Fay, Jin, et al., 2012). In *Sorghastrum*, CO_2 effects were mediated by relative species AGB, while in *Solidago* they were mediated by soil moisture and canopy light level. Thus, different potential causal mechanisms may underlie bivariate relationships (i.e., Figure 1) of CO_2 enrichment to flowering culm count, which are ordinarily interpreted to primarily represent increased photosynthetic efficiency (Ainsworth & Rogers, 2007).
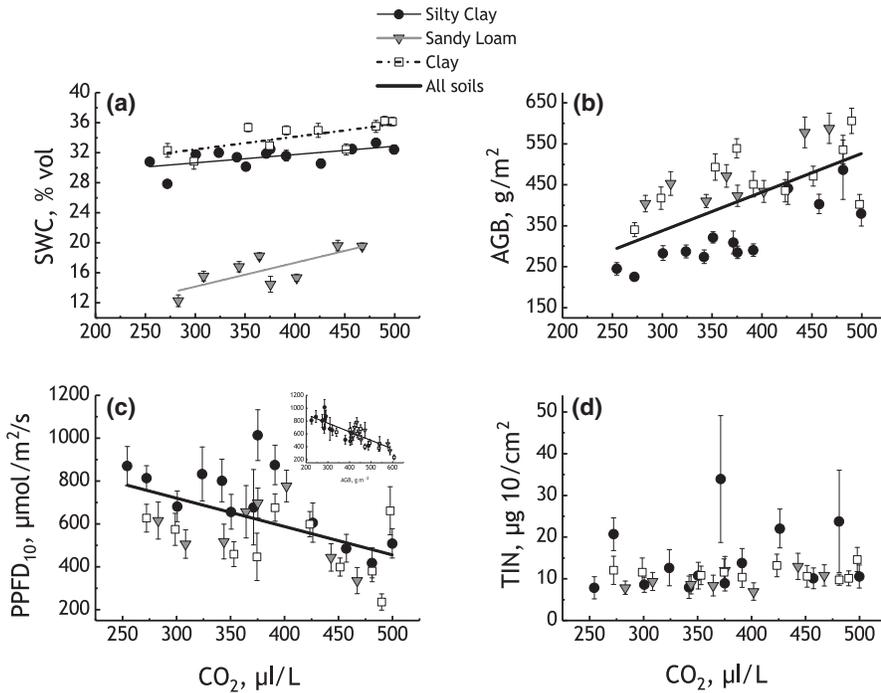
FIGURE 3 (a) Volumetric soil water content for 0–40 cm depth (SWC_{40}), (b) total aboveground biomass (AGB), (c) photosynthetic photon flux density (PPFD) at 10 cm height, and (d) soil total inorganic nitrogen (TIN, $NO_3 + NH_4$) in soil solution in relation to CO_2 concentration on silty clay, sandy loam, and clay soils. Each datum represents the mean ± *SE* of 10 years of CO_2 treatment. Lines indicate significant linear regression relationships across soils or for individual soils. (c) inset is PPFD as a function of total AGB
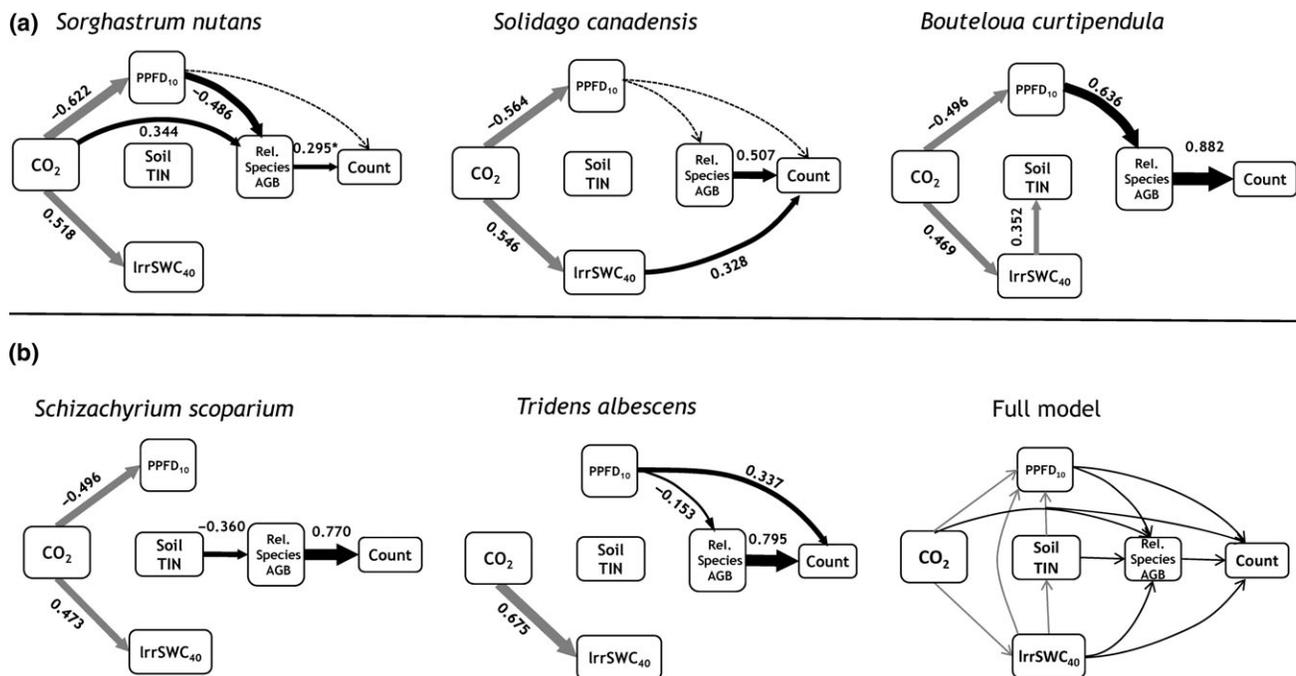

FIGURE 4 Structural equation models identifying combined effects of CO_2 enrichment, canopy light level at 10 cm height ($PPFD_{10}$), the log response ratio of volumetric soil water content to 40 cm depth ($IrrSWC_{40}$), and soil resin-available total inorganic nitrogen (TIN) on relative species aboveground biomass (Rel. Species AGB; species AGB/totalAGB) and flowering culm counts (Count). (a) Species with pathways linking CO_2 to flowering culm counts. (b) Species lacking paths linking CO_2 to flowering culm counts, and the full model showing all hypothesized paths. Gray-shaded arrows denote interactions among drivers of vegetative growth and flowering, black arrows denote driver effects on plants, and dashed arrows denote non-significant paths that combined with other paths to produce significant total effects (Table 1). Path values are standardized coefficients. *Denotes $p = .19$ for this path although the corresponding linear regression (Figure 2a) had $p < .0001$. PPFD, photosynthetic photon flux density

Similarly, the SEMs for species exhibiting weak or absent CO_2-flowering culm count relationships also exhibited different patterns of effects. In *Bouteloua*, the SEM contained no CO_2-flowering culm count effect despite resolving a CO_2 effect on relative species AGB because of the decreasing strength of effects acting in series. In *Schizachyrium* and *Tridens*, there were no paths emanating from CO_2.

TABLE 2 Direct and indirect effects of CO_2 enrichment, soil moisture (SWC_{40}), canopy light level ($PPFD_{10}$), and soil total inorganic nitrogen (TIN) on inflorescence production and relative species aboveground biomass from structural equation models (Figure 4)

Species	CO₂ Direct	CO₂ Indirect	SWC₄₀ Direct	SWC₄₀ Indirect	PPFD₁₀ Direct	PPFD₁₀ Indirect	TIN Direct	TIN Indirect
Standardized effects on inflorescence production								
Sorghastrum nutans	0.296	**0.341**	0.058	0.007	*−0.191*	*−0.142*	−0.130	−0.066
Solidago canadensis	0.106	**0.415**	0.328	−0.126	*−0.231*	*−0.114*	−0.154	−0.014
Bouteloua curtipendula	−0.009	−0.232	0.100	0.105	*−0.238*	**0.561**	−0.183	0.101
Schizachyrium scoparium	0.147	−0.080	−0.015	−0.190	−0.186	0.122	−0.061	**−0.289**
Tridens albescens	0.051	−0.139	0.148	0.279	*0.337*	−0.121	−0.169	−0.214
Standardized effects on relative species aboveground biomass								
Sorghastrum nutans	**0.344**	0.333	0.081	−0.021	**−0.486**	—	−0.158	−0.029
Solidago canadensis	0.218	0.049	−0.120	−0.022	−0.224	—	−0.020	−0.002
Bouteloua curtipendula	−0.191	−0.229	0.145	0.040	**0.636**	—	0.167	−0.090
Schizachyrium scoparium	−0.019	−0.197	−0.250	−0.001	0.159	—	**−0.360**	0.029
Tridens albescens	−0.463	0.341	0.355	0.087	**−0.153**	—	−0.194	0.043

Bold font denotes significant effects. Italicized font denotes a significant sum of direct + indirect effects (=total effect).

Schizachyrium was distinct in that its SEM included no relationships of relative species AGB and flowering with $PPFD_{10}$ or SWC_{40}, suggesting independence from variation in canopy light level and soil moisture. Instead, the SEM indicated that *Schizachyrium* relative AGB increased with decreasing soil inorganic nitrogen. This finding is the opposite of that expected if *Schizachyrium* AGB was limited by nitrogen availability, and may indicate the presence of some unmeasured effect that co-varied with inorganic nitrogen availability.

$PPFD_{10}$ emerged as an important mediator of flowering in these species, and was directly or indirectly related to relative species AGB and flowering culm count in four of the five species. $PPFD_{10}$ is a measure of canopy light availability, but may also represent mechanisms such as competition for soil nutrients or water because of its strong relationship to total AGB. The combination of mechanisms represented by $PPFD_{10}$ can explain the contrasting light availability-relative species AGB-flowering associations in *Sorghatrum* and *Bouteloua*, the two most abundant species in these communities. *Sorghastrum* is a tallgrass that increases strongly as a proportion of community biomass with CO_2 enrichment (Polley et al., 2012a, 2012b), and likely becomes a stronger competitor while contributing to lower canopy light levels. In contrast, *Bouteloua* is a shorter-statured midgrass, and competition from *Sorghastrum*, mediated in part by light levels, may explain its decline with increasing CO_2.

4.2 | Prediction 2

Our finding that allocation to flowering was weakly related or unrelated to CO_2 enrichment in these species contrasts with previous findings that CO_2 enrichment reduced allocation to flowering in native herbaceous perennials (Wang et al., 2015). Allocation expresses the outcome of competition among sinks within plants, such as between vegetative and reproductive growth, for limiting resources (Bazzaz et al., 1987; Harper, 1977; McGinley & Charnov, 1988). For example, in crop species grains or fruits are strong C sinks, and CO_2 enrichment has been shown to increase reproductive allocation (Wang et al., 2015), likely by reducing C limitation of these sinks. In the native perennial species studied here, the weak evidence for CO_2 effects on allocation suggests across these ranges of CO_2 and resource levels, flowering and growth were similarly resource limited (Bazzaz et al., 1987; Harper, 1977; McGinley & Charnov, 1988). Thus, the combined effects of CO_2 enrichment and resource availability affected flowering primarily by altering the constraints on species AGB rather than constraints on allocation to flowering.

4.3 | Contributions of soils

CO_2 effects on soil moisture and soil inorganic nitrogen continued patterns seen earlier in the experiment. Resin-available soil inorganic nitrogen continued to be unrelated to CO_2 (Fay, Jin, et al., 2012), suggesting that this measure of soil N availability has not decreased with CO_2 enrichment in later years of the experiment, as might be expected (Gill et al., 2002; Luo et al., 2004; Reich et al., 2006). Soil-specific patterns in species flowering responses to CO_2 were generally consistent, with one exception arising from underlying responses on the two clay soils (Table S2). The lack of CO_2–flowering relationships on the sandy loam soil is surprising, given its stronger CO_2 response and lower water holding capacity compared to the clay soils. However, more coarse-textured soils also may have greater plant availability of soil moisture at lower soil water contents

compared to more fine-textured soils (Brady & Weil, 2002; Fay, Jin, et al., 2012).

With the exception of *Solidago*, CO_2 effects on soil moisture in the SEMs did not carry through to relative species AGB or flowering culm counts. The absence of soil moisture–flowering relationships in SEMs for the remaining species may result from several factors, including a relatively narrow range of SWC_{40} change with CO_2 on the two clay soils compared to the change in $PPFD_{10}$ (Figure 3). However, in *Solidago*, as a C_3 species flowering may be more coupled to increased soil moisture and improved carboxylation efficiency at enriched CO_2 than in the C_4 grasses in this study (Ainsworth & Rogers, 2007).

4.4 | Other fitness components

Inflorescence production is the initial step in the components determining plant fitness, and may be the main component in the effects of CO_2 enrichment on seed production in grasslands (HilleRisLambers et al., 2009). However, conclusions on the ultimate fitness consequences of these findings for flowering culm production are limited because $CO_2 \times$ resource interactions will depend on effects on subsequent fitness components (Edwards et al., 2001; Hovenden et al., 2007). Other things being equal, an increase or decrease in flowering culm production should result in concomitant changes in seed production, dispersal, and seedling numbers. However, if CO_2 independently affects seed numbers, size, or germination, CO_2 effects on inflorescence production could be amplified or diminished accordingly. CO_2 effects on these fitness components vary, and warrant further study. Also, the final outcome for plant fitness is also related to interactions with factors not directly related to the production of flowers or seeds, like the effects of CO_2 enrichment on litter production (Loydi, Eckstein, Otte, & Donath, 2013) and on the extent of co-occurring changes in phenology (Badeck et al., 2004), precipitation (Walck, Hidayati, Dixon, Thompson, & Poschlod, 2011), or microsite availability (Peters, 2002) that affect the suitability of habitat for successful reproduction.

When considered across soil types representing realistic topoedaphic variation in grassland landscapes, inflorescence production in these tallgrass prairie species was primarily a function of their abundance, as indicated by their AGB, and was associated with CO_2 concentration, light availability, soil moisture, and soil inorganic nitrogen in species-specific combinations. Heterogeneity in CO_2 and resources across soils and varying species responses in multi-species communities may result in more variable or opposing responses in flowering from those expected based on studies on a single soil or in monoculture. Understanding the controls on species abundance under CO_2 enrichment is a critical step in predicting the ability of populations of grassland species to adapt to future environmental change, and therefore their probability of long-term persistence.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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