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Peter B. Reich

University of Minnesota, St. Paul, MN

David Tilman

University of Minnesota, St. Paul, MN

Joseph Craine

University of California, Berkeley, CA

David Ellsworth

Brookhaven National Laboratory, Upton, NY

Mark G. Tjoelker

University of Minnesota, St. Paul, MN

See next page for additional authors

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Authors

Peter B. Reich, David Tilman, Joseph Craine, David Ellsworth, Mark G. Tjoelker, Johannes M. N. Knops, David A. Wedin, Shahid Naeem, Dan Bahauddin, Jenny Goth, Wendy Bengtson, and Tali D. Lee

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

Peter B. Reich¹, David Tilman², Joseph Craine³, David Ellsworth⁴, Mark G. Tjoelker¹, Jean Knops⁵, David Wedin⁶, Shahid Naeem⁷, Dan Bahauddin¹, Jenny Goth¹, Wendy Bengtson¹, Tali D. Lee¹

¹Department of Forest Resources, University of Minnesota, St. Paul, MN 55108 USA; ²Department of Ecology, Evolution and Behaviour, University of Minnesota, St. Paul, MN 55108 USA; ³Department of Integrative Biology, University of California, Berkeley, CA 94720 USA; ⁴Department of Environmental Science, Brookhaven National Laboratory, Upton, NY 11973 USA; ⁵School of Biological Sciences, University of Nebraska, Lincoln, NE 68583 USA; ⁶School of Natural Resource Sciences, University of Nebraska, Lincoln, NE 68583 USA; ⁷Department of Zoology, University of Washington, Seattle, WA 98195 USA

Summary

Author for correspondence:
Peter B. Reich
Tel: +1 612 624 4270
Fax: +1 612 625 5212
Email: preich@forestry.umn.edu

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- To evaluate whether functional groups have a similar response to global change, the responses to CO₂ concentration and N availability of grassland species from several functional groups are reported here.
- Sixteen perennial grassland species from four trait-based functional groups (C₃ grasses, C₄ grasses, non-leguminous forbs, legumes) were grown in field monocultures under ambient or elevated (560 μmol mol⁻¹) CO₂ using free-air CO₂ enrichment (FACE), in low N (unamended field soil) or high N (field soil +4 g N m⁻² years⁻¹) treatments.
- There were no CO₂ × N interactions. Functional groups responded differently to CO₂ and N in terms of biomass, tissue N concentration and soil solution N. Under elevated CO₂, forbs, legumes and C₃ grasses increased total biomass by 31%, 18%, and 9%, respectively, whereas biomass was reduced in C₄-grass monocultures. Two of the four legume species increased biomass and total plant N pools under elevated CO₂, probably due to stimulated N-fixation. Only one species markedly shifted the proportional distribution of below- vs aboveground biomass in response to CO₂ or N.
- Although functional groups varied in responses to CO₂ and N, there was also substantial variation in responses among species within groups. These results suggest that current trait-based functional classifications might be useful, but not sufficient, for understanding plant and ecosystem responses to elevated CO₂ and N availability.

Key words: Functional groups, elevated carbon dioxide, nitrogen, N availability, grasses, forbs, legumes, biomass.

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Introduction

Generalizing about responses of different plant species to elevated atmospheric CO₂ concentrations or N deposition remains an elusive goal in global change biology. Given the wide variety of species, any kind of grouping that simplifies the variation among species while providing predictive power will

significantly advance the field. Based on well-known differences among species in important intrinsic traits, a variety of hypotheses have been developed about potential functional group differences in response to CO₂ and N enrichment.

1) Intrinsic differences in photosynthetic biochemistry should lead to markedly greater responsiveness to elevated CO₂ for C₃ than C₄ plants (H₁). Although supported by

theory and early field studies (Percy & Ehleringer, 1984; Curtis *et al.*, 1989), more recent studies suggest such differences may be less pronounced (Owensby *et al.*, 1993; Wand *et al.*, 1999), especially during dry periods.

2) Productivity in N-fixing legumes may be stimulated by elevated CO₂ more than in nonfixers (H₂), because the former should be less N-limited. It has long been known that elevated CO₂ stimulates legume growth and N₂ fixation (Finn & Brun, 1982; Zanetti *et al.*, 1996). N-fixing species have often shown a stronger biomass response to elevated CO₂ than nonfixing species (Soussana & Hartwig, 1996; Clark *et al.*, 1997; Hebeisen *et al.*, 1997; Lüscher & Nösberger, 1997, 1998; Schenk *et al.*, 1997), although little work to date has been on wild species at naturally low levels of N availability.

3, 4) A series of related hypotheses posit that increases in CO₂ or N supply should lead to a more pronounced growth increase in species of given strategies, habitats, or growth rates. For our study we propose the hypothesis (H₃) that C₃ grasses considered more disturbance adapted and nitrophilic, should respond more to increase in N supply than C₄ grasses (cf Wedin & Tilman, 1996). Moreover, being less N-limited, the legumes should also be less responsive to N addition than the non-fixers (H₄).

5) In addition to understanding the effects of elevated CO₂ or N singularly, given the potential for the CO₂ fertilization effect to be modulated by N-supply, it is important to study species and functional group responses to combinations of these two elements. Many ecosystem models theorize that CO₂ responses are constrained by N limitation (but see Cannell & Thornley, 1998) and actual evidence is mixed (Larigauderie *et al.*, 1988; Owensby *et al.*, 1994; Leadley & Körner, 1996; Lloyd & Farquhar, 1996; Poorter *et al.*, 1996; Volin & Reich, 1996; Zak *et al.*, 2000). If the CO₂ response of species is generally N-limited, then nonlegumes, without the ability to fix atmospheric N and therefore modulate their own N supply, should demonstrate smaller responses to elevated CO₂ than legumes (see H₂) and be more likely to show a CO₂ × N interaction (H₅).

6) For individually grown plants, usually in their first year, root fraction (root biomass as a fraction of total biomass) adjusted for ontogenetic drift is unaffected by CO₂ (Curtis & Wang, 1998; Reich, 2001) and is lower under enhanced N supply (Poorter & Nagel, 2000; Reich, 2001). Data for older plants or stands are rare. If assemblages over multiple years behave similarly as young, individual plants, one might hypothesize that elevated CO₂ would have no effect (H_{6a}) and N addition a decreasing effect (H_{6b}) on root fraction in our experiment. Since root fraction incorporates both allocation and turnover, neither of which are well documented in the field (Reich, 2001), these hypotheses are proposed as null models.

7) Effects of treatments on biomass and physiology should be reflected in plot-scale resource availability. Assuming that greater CO₂ and N supply both lead to increased biomass, we hypothesize (H₇) that soil solution N concentration and

percentage soil water should decrease under both treatments. However, increased N supply should compensate for increased N uptake, minimizing the decline in soil N compared with that under elevated CO₂ (H_{7a}). Under elevated CO₂, reduced leaf level water loss could minimize the decline in percentage soil water compared to that experienced in the high N treatment (H_{7b}).

Although there are an increasing number of tests of the CO₂ × N interaction hypothesis in general and of the functional group-related hypotheses raised above, few have been done in field settings where both root and shoot processes can be quantified for more than a small number of species. To help fill this gap, we addressed these issues using an experiment comprising 16 grassland species from four functional groups grown in 128 monoculture plots that is a part of a larger experiment (BioCON) designed to test interactions among species diversity, elevated CO₂ and N deposition (Reich *et al.*, 2001). In particular, we assessed whether functional groups, growing in monoculture plots in a free-air CO₂ enrichment (FACE) experiment, differed in their acquisition and use of C, N and water in response to combined treatment with elevated CO₂ and N deposition.

Materials and Methods

The BioCON (Biodiversity, CO₂ and N) experiment (Reich *et al.*, 2001) (<http://www.swan.lter.umn.edu/biocon/>) is located at the Cedar Creek Natural History area, a National Science Foundation, Long-Term Ecological Research site in Minnesota, USA (lat. 45° N, Long. 93° W). The region has a continental climate with cold winters, warm summers (mean January and July temperatures of -11 and 22°C), and precipitation averaging 660 mm y⁻¹. The soils are derived from a glacial outwash sand plain and are sandy and nitrogen poor. Plots were established on a secondary successional grassland after removing the previous vegetation.

Our study included 128 individual monoculture plots (each 2 × 2 m), a subset of all plots, distributed nearly equally among six 20-m diameter experimental areas (rings). In three elevated CO₂ rings, a free-air CO₂ enrichment (FACE) system (Lewin *et al.*, 1994) was used during the 1998 and 1999 growing seasons to maintain the CO₂ concentration at 560 μmol mol⁻¹. Three ambient rings (368 μmol mol⁻¹ CO₂) were treated identically but without additional CO₂. The experimental treatments were arranged in complete factorial combination of CO₂ (ambient or elevated), species (a total of 16, four from each of four functional groups), and N level (low and high) for a 2 × 16 × 2 design with two replicates. Each plot was planted in 1997 with 12 g m⁻² of seed. The design consisted of a split-plot arrangement of treatments in a completely randomized design. CO₂ treatment is the whole-plot factor and is replicated three times among the six rings. The subplot factors of species identity and N treatment were randomly assigned and replicated in individual plots among the six rings. CO₂ was added in

elevated treatments during all daylight hours from April 9 to October 16, 1998, and from April 20 to November 9, 1999. Tests found no direct effect of elevated CO₂ on dark respiration (Tjoelker *et al.*, 2001). During CO₂ enrichment periods, 1-min averages were within 10% of the target concentration 94% of the time in 1998 and 95% of the time in 1999. Beginning in 1998, the plots assigned to the high N treatment were amended with 4 g N m⁻² yr⁻¹, applied over three dates each year, while the low N treatment soil was unamended. During the two growing seasons of treatments during this study, no severe dry periods occurred.

The 16 perennial species used in this study were all native or naturalized to the Cedar Creek Natural History Area. They include four C₄ grasses (*Andropogon gerardii* Vitman, *Bouteloua gracilis*, *Schizachyrium scoparium* (Michaux) Nash, *Sorghastrum nutans* (L.) (Nash), four C₃ grasses (*Agropyron repens* (L.) Beauv., *Bromus inermis* Leysser, *Koeleria cristata* Pers, *Poa pratensis* L.), four N-fixing legumes (*Amorpha canescens* Pursh, *Lepedeza capitata* Michaux, *Lupinus perennis* L., *Petalostemum villosum* Nutt.) and four nonN-fixing herbaceous species (*Achillea millefolium* L., *Anemone cylindrica* A. Gray, *Asclepias tuberosa* L., *Solidago rigida* L.). Species hereafter are referred to by their genus. Monocultures of all species were replicated twice at all four combinations of CO₂ and N levels. Plots were regularly weeded to remove unwanted species. In June and August of 1998 and 1999 we assessed above- and belowground (0–20 cm) biomass and soil solution N concentrations (extracted using 0.01 mol KCl). For most analyses in this paper we use the mean values per plot from these four harvests. A 10 × 100 cm strip was clipped at just above the soil surface, all matter was collected, sorted to live material and senesced litter, dried and weighed. Roots were sampled at 0–20 cm depth using three 5-cm cores in the area used for the aboveground biomass clipping. Roots were washed, sorted into fine (< 1 mm diameter) and coarse classes and crowns, dried and weighed. Volumetric soil moisture levels (0–20 cm depth) were assessed periodically (on 18 sampling periods) in all plots in 1998 and 1999 using time-domain reflectometry (Baker, 1990). A composite sample was taken from aboveground and belowground biomass from each plot from the August harvests of each year, ground and analysed for N using a CHN analyser (Carlo-Erba Strumatzione, Milan, Italy). To estimate total plant N stocks we multiplied whole plant percentage N (averaged across years) by the mean whole plot biomass (averaged from all harvests).

Data analysis

ANOVA was used in two complementary ways, including either species or functional group as a treatment effect. In ANOVA all treatment effects were considered fixed. Using *F*-tests, the effect of CO₂ (1 df) was tested against the random effect of ring nested within CO₂ (4 df). The main effects of functional group (3 df) was tested against the random effect of species nested within functional group (12 df). The main

effects of species (15 df), and N (1 df), and interactions between CO₂, N, and either functional group or species were tested against the residual error. Additionally, to explicitly test hypotheses about preplanned specific functional group contrasts (e.g. C₃ vs C₄), their effects were partitioned into single-degree-of-freedom contrasts. We also evaluated the proportional distribution of biomass aboveground vs belowground. Since this is a plot-scale measure for assemblages that had been developing in the field for three seasons it should not be taken as a measure of allocation. Instead, it reflects the balance between allocation and turnover (Reich, 2001). We also used separate and same slopes regression to test for slope and intercept differences among treatments in the relationships between soil resources (e.g. % soil water or N) and fine root biomass. We used repeated measures ANOVA to test whether responses to CO₂ or N varied among times of year (June vs August), among years, or among all four harvests. There were no interactions between treatments and time, hence all results are presented averaged across harvests and years. All statistical analyses were made using JMP 4.0.1 software.

Results

Functional groups differed significantly in shoot, root and total biomass (Tables 1, 2; Fig. 1), with C₃ grass monocultures highest and legumes lowest. For total biomass, averaged across all species or functional groups, there were marginally significant main effects of CO₂ (+11%, mean of enhancement) and N (+7%) (Tables 2, 3; Fig. 1) and there were no significant CO₂ × N interactions (Tables 2, 3). In fact, there were no significant CO₂ × N interactions, or significant three-way interactions involving CO₂ and N, for any of the measured variables in this study (refuting H₅), and hence responses to CO₂ and N will be presented separately, pooled across levels of the other variable.

Biomass response to elevated CO₂

There were marked differences among functional groups in terms of biomass response to CO₂ (Tables 1, 2; Figs 1, 2). Forbs, legumes, and C₃ grasses increased total biomass by 31%, 18%, and 9%, respectively, under elevated CO₂ whereas C₄ grass monocultures had 3% lower total biomass. The interaction term was significant (*P* < 0.10) for the CO₂–group interaction and more so (*P* < 0.002) for the C₃ vs C₄ contrast (Table 2), indicating that the C₄ grasses did have less enhancement of biomass than C₃ species in general, supporting (H₁). There was no evidence that the N-fixing legumes responded more positively to elevated CO₂ than the nonfixing C₃ species (Figs 1, 2; Tables 1, 2), refuting (H₂). The effects of CO₂ on biomass, and differences in response among functional groups, were largely manifest belowground (Tables 1, 2).

There was substantial variation in response to elevated CO₂ among species, shown by significant species–CO₂ interactions

Table 1 Means of belowground and aboveground biomass of species under contrasting CO₂ and N treatments for 128 plots. Values shown are for 4 plots per species at each CO₂ or N level, pooled across treatments otherwise and averaged over four harvests in two years. The mean standard errors of the adjusted least squares means (LSM) for belowground and aboveground biomass, respectively, were 59 and 21 g m⁻² for species and 103 and 14 for functional groups

Functional group	Species	Belowground biomass (g m ⁻²)				Aboveground biomass (g m ⁻²)			
		Ambient CO ₂	Elevated CO ₂	Low N	High N	Ambient CO ₂	Elevated CO ₂	Low N	High N
C ₄ grass	<i>Andropogon gerardii</i>	575	525	543	557	261	283	263	282
	<i>Bouteloua gracilis</i>	472	562	471	564	253	200	209	244
	<i>Schizachyrium scoparium</i>	359	349	293	415	177	153	133	196
	<i>Sorghastrum nutans</i>	569	513	614	468	248	221	199	270
	Mean	493	489	480	502	236	215	204	247
C ₃ grass	<i>Agropyron repens</i>	806	1114	883	1037	308	307	282	333
	<i>Bromus inermis</i>	828	755	750	834	306	281	253	333
	<i>Koeleria cristata</i>	645	714	666	692	253	225	194	284
	<i>Poa pratensis</i>	991	1058	929	1120	168	200	123	245
	Mean	817	910	807	920	256	252	210	299
Forb	<i>Achillea millefolium</i>	700	1014	944	770	268	293	266	295
	<i>Anemone cylindrica</i>	183	288	324	147	36	53	60	29
	<i>Asclepias tuberosa</i>	97	172	91	179	11	14	12	13
	<i>Solidago rigida</i>	597	690	706	581	315	386	328	372
	Mean	395	541	516	419	160	185	167	179
Legume	<i>Amorpha canescens</i>	172	160	208	124	59	26	59	26
	<i>Lespedeza capitata</i>	274	407	315	366	67	153	143	77
	<i>Lupinus perennis</i>	216	303	270	250	279	383	339	323
	<i>Petalostemum villosum</i>	135	122	118	140	142	39	27	154
	Mean	200	246	226	220	137	151	143	145

Table 2 ANOVA summary for biomass, N, and soil water measures in 128 plots

Parameter	R ²	CO ₂ × N × Functional group analyses							
		CO ₂	N	Group	CO ₂ × Group	CO ₂ × (C ₃ vs C ₄)	CO ₂ × C ₃ N-fixer vs C ₃ nonfixer	N × group	N vs N-fixer vs nonfixer
Total biomass	0.88	0.05	0.10	0.02	0.10	0.002	0.04	0.001	0.08
Belowground biomass	0.87	0.08	0.77	0.006	0.14	0.005	0.02	0.007	0.67
Aboveground biomass	0.84	0.47	0.0003	0.51	0.30	0.14	0.77	0.01	0.0006
Root fraction	0.65	0.40	0.02	0.39	0.70	0.31	0.72	0.92	0.36
Total plant N pool	0.80	0.27	< 0.0001	0.18	0.51	0.18	0.65	0.0006	< 0.0001
Belowground percentageN	0.85	0.30	0.03	0.002	0.38	0.19	0.13	0.01	0.0007
Aboveground percentageN	0.89	0.03	0.005	0.07	0.02	< 0.0001	0.08	0.002	0.0002
% soil water	0.70	0.60	0.0003	0.11	0.11	0.65	0.11	0.009	0.15
Soil nitrate concentration	0.69	0.08	0.001	0.002	0.03	0.01	0.18	0.009	0.47
Soil N concentration	0.74	0.22	0.0005	0.002	0.28	0.07	0.71	0.03	0.84

R² shown for whole model. *P*-values shown for main effects and interactions (bolded when *P* < 0.10). Interactions involving CO₂ × N were not significant at *P* < 0.10) and hence are not shown. Group, functional group contrasts of the four groups; C₃ vs C₄, C₄ grasses vs all other C₃ species; C₃ N-fixer vs C₃ nonfixer, legumes vs forbs plus C₃ grasses; N-fixer vs nonfixer, legumes vs all other nonfixer species.

Fig. 1 Total biomass (aboveground plus belowground, 0–20 cm depth) (g m^{-2}) of 16 species as affected by elevated vs ambient CO_2 treatments (pooled across N treatments) and by high N (addition of $4 \text{ g N m}^{-2} \text{ yr}^{-1}$) vs unamended soil treatments (pooled across CO_2 treatments). Top panel: grey columns, elevated CO_2 ; black columns, ambient CO_2 . Bottom panel: grey columns, low N; white columns, high N. Each value is a mean of four harvests (June and August in each of 1998 and 1999) for 4 plots per species-treatment combination. The mean standard errors of the adjusted least squares means (LSMs) were 66 g m^{-2} for species. Statistical details provided in Tables 2 and 3.

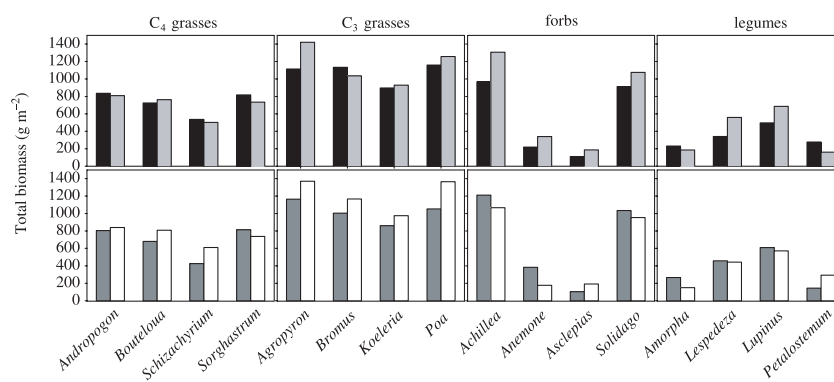


Table 3 ANOVA summary for biomass, N, and soil water measures in 128 plots

Parameter	$\text{CO}_2 \times \text{N} \times \text{Species}$ analyses					
	R^2	CO_2	N	Species	$\text{CO}_2 \times \text{Species}$	N \times Species
Total biomass	0.94	0.06	0.07	< 0.0001	0.01	0.01
Belowground biomass	0.93	0.11	0.73	< 0.0001	0.04	0.02
Aboveground biomass	0.94	0.53	< 0.0001	< 0.0001	0.0009	0.0004
Root fraction	0.87	0.33	0.004	< 0.0001	0.02	0.003
Total plant N pool	0.91	0.29	< 0.0001	< 0.0001	0.02	0.0003
Belowground percentageN	0.91	0.34	0.04	< 0.0001	0.98	0.05
Aboveground percentageN	0.96	0.01	0.001	< 0.0001	0.01	< 0.0001
% soil water	0.82	0.58	0.0005	< 0.0001	0.68	0.13
Soil nitrate concentration	0.86	0.09	0.0004	< 0.0001	0.002	0.12
Soil N concentration	0.87	0.15	0.0003	< 0.0001	0.01	0.29

R^2 shown for whole model. P -values shown for main effects and interactions (bolded when $P < 0.10$), except those involving $\text{CO}_2 \times \text{N}$ which were not significant at $P < 0.10$.

for shoot, root and total biomass (Tables 1, 3). Seven species increased total biomass under elevated CO_2 by at least 100 g m^{-2} , including three forbs, two C_3 grasses and two legumes (Fig. 1). The largest responses to elevated CO_2 were shown by a forb, *Achillea* ($+339 \text{ g m}^{-2}$, $+35\%$), a C_3 grass, *Agropyron* ($+307 \text{ g m}^{-2}$, $+28\%$) and two legumes, *Lespedeza* ($+218 \text{ g m}^{-2}$, $+64\%$) and *Lupinus* ($+190 \text{ g m}^{-2}$, $+38\%$), all of which were statistically significant ($P < 0.05$) using post hoc tests. However, some species within all three of these C_3 functional groups also had modest or minimal responses to elevated CO_2 (Fig. 1).

Biomass response to N addition

Functional groups responded differently ($P < 0.01$) to N treatment in terms of both aboveground and belowground biomass (Tables 1, 2). At high N, C_3 grasses showed the greatest increase in total biomass of all functional groups (Tables 1, 2; Figs 1, 2), supporting (H_3), and the legumes responded less positively in general than the nonfixers, supporting (H_4). However, the forb group also failed to respond to high N with increased biomass production. Moreover, except within

the C_3 grass group, variation in biomass response to N among species within functional groups was substantial, shown by (Table 1, Fig. 1).

There were significant species–N interactions for shoot, root and total biomass (Tables 1, 3; Fig. 1). Seven species increased total biomass under high N by at least 140 g m^{-2} , including all four C_3 grasses, two C_4 grasses, and a legume, with *Poa* ($+313 \text{ g m}^{-2}$, $+30\%$), *Agropyron* ($+206 \text{ g m}^{-2}$, $+18\%$) and *Schizachyrium* ($+163 \text{ g m}^{-2}$, $+37\%$) showing the largest increases.

Variation among functional groups and species

How do differences among functional groups compare with differences among species? We assessed the coefficient of variation (CV) of functional group and species means, and of the responses to treatments, for many variables. Results for total biomass are representative of the general trends. The CV among groups for total biomass at each of the four CO_2 and N treatment combinations was slightly less than the CV for all species assessed collectively (Table 4). However, the CV among species within groups varied extremely among groups, from as low as 15–20% for C_3 grasses to as high as 70–80%

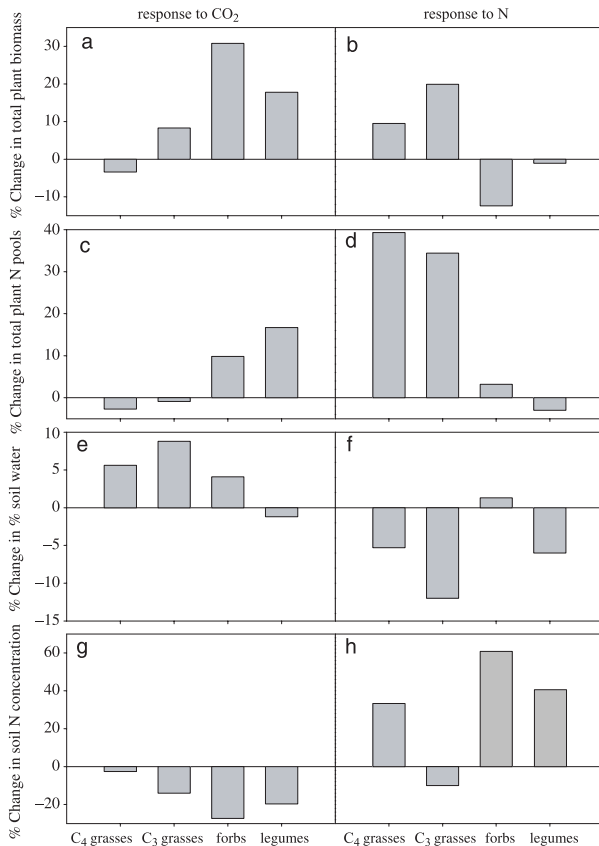


Fig. 2 Proportional response of total biomass, plant N pool, percentage soil water, and soil solution N concentration to CO_2 and N treatments (otherwise pooled) on average for species in four functional groups. See Table 2 for statistics.

for the forbs. The response of biomass to CO_2 also had a lower CV among groups (102%) than among all species (187%).

Proportional biomass distribution under elevated CO_2 and N treatments

Functional groups did not differ significantly in the proportion of total biomass found belowground, that is root fraction (Tables 2, 5). The C_4 grasses had root fractions between 67% and 69%, the C_3 grasses had root fractions between 74% and 86%, and species within other functional groups varied

widely. Among all species, *Lupinus* had the lowest root fraction (43%) but other legumes had intermediate or high root fractions (69–82%).

Root fractions under elevated CO_2 (0.74 on average) were not significantly different than under ambient CO_2 (0.72). Root fraction was lower ($P < 0.005$) in the high N (0.71) than the low N treatment (0.75). These results support H_6 . Root fraction was unrelated to total biomass. There were no significant functional group \times N or group– CO_2 interactions for root fraction (Table 2), but there were significant species \times CO_2 and species–N interactions (Table 3). Thus, functional groups did not show different biomass distribution response to CO_2 or N, but species did. Six species, representing all four functional groups, had lower root fraction under high N (Table 5). *Petalostemum* had a root fraction of 0.84 under low N and 0.54 under high N, while another legume species (*Lespedeza*) had greater root fraction under high N (0.81) than low N (0.72). Four species, one from each functional group, had higher root fraction under elevated CO_2 (Table 5). *Petalostemum* was again the most plastic in root fraction, showing root fraction of 0.55 in ambient CO_2 and 0.82 under elevated CO_2 . Again, *Lespedeza* showed an opposite response to most other species, having lower root fraction under elevated than ambient CO_2 . Thus, species were quite varied in the magnitude of biomass distribution responses, with no relation to functional group membership, and in fact the two species with the most pronounced and opposite behaviour were both legumes.

Total plant N and tissue N concentration

Averaged across all functional groups or species, the total plant N pool was significantly higher with N addition and unchanged with elevated CO_2 (Tables 2, 3). Thus, despite greater root biomass under elevated CO_2 , total cumulative N uptake did not increase, and hence there was substantial dilution of N in biomass (see below). Species differed markedly ($P < 0.001$) in how plant N pools responded to CO_2 treatments, however (Table 3, Fig. 3). *Lespedeza* and *Lupinus* had large increases in total plant N (of roughly 40–60%, +3.3–4.0 g N m^{-2}) with increased CO_2 , whereas most other species had similar total N in the elevated vs ambient CO_2 treatment. Response of total plant N pools to N treatment differed ($P < 0.05$) by functional groups; with forbs showing minimal increase, whereas both

	Low N		High N	
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
C_3 grass spp.	11.5	12.6	12.6	21.8
C_4 grass spp.	25.7	24.4	12.0	20.1
Forb spp.	85.3	70.4	76.4	83.7
Legume spp.	43.3	70.3	41.0	62.8
All species	53.8	51.4	55.1	58.5
Functional groups	39.9	36.8	52.7	46.7

Table 4 Coefficient of variation of total biomass per plot among species within functional groups, among all species, and among functional groups, for four treatment combinations of CO_2 and N. Mean values per species or functional group based on averages of all plots in all harvests

Table 5 Means of root fraction (root biomass/total biomass) of species under contrasting CO₂ and N treatments for 128 plots

Functional group	Species	Root fraction (root dm/total plant dm)			
		Ambient CO ₂	Elevated CO ₂	Ambient N	Enriched N
C ₄ grass	<i>Andropogon gerardii</i>	0.69	0.65	0.67	0.67
	<i>Bouteloua gracilis</i>	0.65	0.74	0.69	0.69
	<i>Schizachyrium scoparius</i>	0.68	0.69	0.70	0.68
	<i>Sorghastrum nutans</i>	0.69	0.69	0.75	0.63
	Mean	0.68	0.69	0.70	0.67
C ₃ grass	<i>Agropyron repens</i>	0.73	0.79	0.76	0.75
	<i>Bromus inermis</i>	0.74	0.73	0.76	0.71
	<i>Koeleria cristata</i>	0.72	0.76	0.77	0.71
	<i>Poa pratensis</i>	0.86	0.84	0.89	0.82
	Mean	0.76	0.78	0.80	0.75
Forb	<i>Achillea millefolium</i>	0.71	0.77	0.77	0.72
	<i>Anemone cylindrica</i>	0.84	0.85	0.83	0.86
	<i>Asclepias tuberosa</i>	0.91	0.93	0.91	0.92
	<i>Solidago rigida</i>	0.64	0.64	0.68	0.61
	Mean	0.77	0.80	0.80	0.78
Legume	<i>Amorpha canescens</i>	0.81	0.85	0.82	0.83
	<i>Lespedeza capitata</i>	0.80	0.73	0.72	0.81
	<i>Lupinus perennis</i>	0.43	0.43	0.43	0.43
	<i>Petalostemum villosum</i>	0.55	0.82	0.84	0.54
	Mean	0.65	0.71	0.70	0.65

Values shown are for 4 plots per species at each CO₂ or N level, pooled across treatments otherwise and averaged over four harvests in two years. The mean standard errors of the adjusted LSMs were 0.04 for species and 0.06 for functional groups.

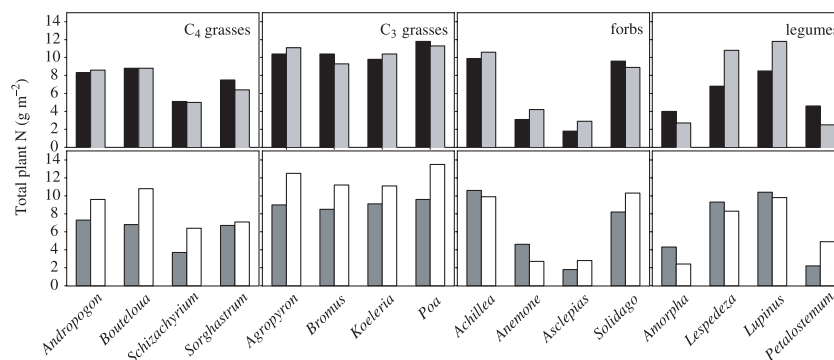


Fig. 3 Means of total plant N pools (g m⁻²) of 16 species under contrasting CO₂ and N treatments. Top panel: grey columns, elevated CO₂; black columns, ambient CO₂. Bottom panel: grey columns, low N; white columns, high N. The mean standard errors of the adjusted LSMs were 0.8 g m⁻² for species. See Tables 2 and 3 for statistics.

grass groups and legumes showed substantial increase in total N (Table 2; Figs 2, 3).

Functional groups differed in tissue percentage N (Tables 2, 6). Root and shoot percentage N responded to treatments differently, in that roots had less pronounced treatment effects and there were fewer interactions between functional groups or species and treatments (Tables 2, 3, 6). For aboveground tissue, CO₂ treatment reduced percentage N by 11% on

average and the high N treatment increased percentage N by 8% on average (pooled across all other sources of variation). Both functional groups and species differed significantly (Tables 2, 3) in response of aboveground percentage N to both treatments. The C₄ grasses did not have lower percentage N under elevated CO₂ (Table 6). All C₃ functional groups tended to have substantially lower tissue percentage N under elevated CO₂ (Table 6), but species varied markedly in this respect.

Table 6 Means of tissue percentage N of species under contrasting CO₂ and N treatments for 128 plots. Values shown are for 4 plots per species at each CO₂ or N level, pooled across treatments otherwise and averaged over August harvests in 1998 and 1999

Functional group	Species	Aboveground % N				Belowground % N				Total % N			
		Amb CO ₂	Elev CO ₂	Low N	High N	Amb CO ₂	Elev CO ₂	Low N	High N	Amb CO ₂	Elev CO ₂	Low N	High N
C ₄ grass	<i>Andropogon gerardi</i>	1.09	1.15	0.93	1.31	0.95	1.00	0.88	1.07	0.98	1.05	0.90	1.13
	<i>Bouteloua gracilis</i>	1.41	1.33	1.11	1.63	1.14	1.07	0.94	1.26	1.24	1.16	1.01	1.40
	<i>Schizachyrium scoparium</i>	1.01	1.20	1.04	1.17	0.89	0.90	0.82	0.98	0.94	1.01	0.91	1.05
	<i>Sorghastrum nutans</i>	0.91	0.89	0.80	1.00	0.88	0.87	0.82	0.94	0.89	0.89	0.81	0.97
	Mean	1.10	1.13	0.95	1.28	0.97	0.96	0.87	1.06	1.01	1.02	0.90	1.13
C ₃ grass	<i>Agropyron repens</i>	1.26	0.89	0.96	1.19	0.84	0.72	0.72	0.84	0.94	0.73	0.75	0.92
	<i>Bromus inermis</i>	0.91	1.00	0.80	1.10	0.91	0.85	0.87	0.89	0.91	0.89	0.85	0.94
	<i>Koeleria cristata</i>	1.43	1.30	1.20	1.53	0.95	1.03	1.01	0.97	1.12	1.12	1.06	1.18
	<i>Poa pratensis</i>	1.42	1.10	1.17	1.37	0.95	0.86	0.89	0.91	1.03	0.91	0.93	1.01
	Mean	1.27	1.08	1.05	1.30	0.90	0.86	0.87	0.90	1.00	0.92	0.90	1.02
Forb	<i>Achillea millefolium</i>	1.47	1.27	1.35	1.39	0.85	0.71	0.78	0.78	1.06	0.87	0.93	1.00
	<i>Anemone cylindrica</i>	1.89	1.55	1.57	1.88	1.37	1.20	1.19	1.38	1.49	1.30	1.27	1.51
	<i>Asclepias tuberosa</i>	3.44	2.94	3.51	2.86	1.45	1.42	1.49	1.38	1.90	1.65	1.77	1.78
	<i>Solidago rigida</i>	1.07	0.80	0.80	1.08	1.09	0.84	0.79	1.14	1.10	0.79	0.80	1.10
	Mean	1.95	1.65	1.80	1.80	1.20	1.04	1.06	1.18	1.39	1.15	1.19	1.35
Legume	<i>Amorpha canescens</i>	2.70	2.17	2.47	2.40	1.48	1.44	1.40	1.53	1.73	1.55	1.58	1.71
	<i>Lespedeza capitata</i>	2.35	1.95	2.12	2.18	1.89	1.92	1.98	1.83	2.00	1.93	2.02	1.91
	<i>Lupinus perennis</i>	1.52	1.43	1.43	1.52	2.15	2.12	2.29	1.99	1.87	1.78	1.88	1.78
	<i>Petalostemum villosum</i>	2.26	2.25	2.46	2.05	1.35	1.33	1.33	1.35	1.72	1.51	1.61	1.62
	Mean	2.21	1.95	2.12	2.04	1.71	1.70	1.75	1.67	1.83	1.69	1.77	1.75

The mean standard error of the adjusted LSMs was 0.10, 0.09, and 0.08% for aboveground, belowground and total percentage N for species and 0.14, 0.07, and 0.07% for aboveground, belowground and total percentage N for functional groups.

Agropyron, *Amorpha*, *Anemone*, *Asclepias*, *Lespedeza*, and *Poa* (equally representing the three C₃ groups) decreased percentage N by the greatest amounts under elevated CO₂, and most other C₃ species had smaller or negligible decreases. Except for the legumes and *Asclepias*, all other species had modest or marked increases in aboveground percentage N with N addition (Table 6).

Soil water

On average, plots had lower percentage soil water (% SW) in the high N than low N treatments, as hypothesized (H₇) (Tables 2, 3, 7; Fig. 2). Moreover, species and functional groups differed marginally in percentage SW and in how percentage SW changed with CO₂ and N. C₃ grasses had the lowest percentage SW, C₄ grass and forbs intermediate and legumes the highest percentage SW (Table 7), likely reflecting the influence of differential root biomass on water uptake. To sum, groups are different in percentage SW, but apparently mostly because they have different fine root biomass. Among

species, however, differences in percentage SW do not neatly follow differences in total or root biomass—*Koeleria* (a C₃ grass) had the lowest percentage SW and *Lupinus*, *Agropyron* and several other species the highest.

On average, C₃ grasses showed the greatest increase in percentage SW with elevated CO₂ and the greatest decrease in percentage SW at high N (Table 7; Fig. 2). Except for the legumes, all species had slightly or substantially higher percentage SW under elevated than ambient CO₂ (Table 7), even when root and total biomass were higher in the latter treatment (e.g. *Achillea*, *Agropyron*, *Anemone*, *Bouteloua*, *Poa*) (supporting H_{7b}). *Bromus* had the most pronounced ($P = 0.05$) increase in percentage SW under elevated vs ambient CO₂, consistent with its slightly lower biomass under elevated CO₂. For N treatments, *Agropyron* and *Koeleria* (C₃ grasses) both had substantially greater depression of percentage SW (both $P < 0.05$) in high N than other species.

Even accounting for variation in fine root biomass, there were significant differences in percentage SW due to CO₂ and N treatments. When data for all plots were pooled and

Table 7 Means of percentage soil water and soil solution N concentrations for species under contrasting CO₂ and N treatments for 128 plots. Values shown are for 4 plots per species at each CO₂ or N level, pooled across treatments otherwise and averaged over multiple samplings for soil water, and two harvests for soil N, in each of 1998 and 1999. The mean standard error of the adjusted LSMs for percentage soil water was 0.03% for species and 0.2% for functional groups, and 0.1 mg kg⁻¹ for soil N for both species and functional groups

Functional group	Species	% soil water				Soil solution N conc. (mg/kg)			
		Amb CO ₂	Elev CO ₂	Low N	High N	Amb CO ₂	Elev CO ₂	Low N	High N
C ₄ grass	<i>Andropogon gerardii</i>	7.4	7.5	7.7	7.2	0.42	0.38	0.33	0.47
	<i>Bouteloua gracilis</i>	6.5	7.3	7.1	6.8	0.55	0.45	0.39	0.61
	<i>Schizachyrium scoparius</i>	7.6	7.9	7.8	7.6	0.38	0.41	0.31	0.48
	<i>Sorghastrum nutans</i>	7.4	7.5	7.5	7.3	0.20	0.29	0.25	0.25
	Mean	7.2	7.6	7.6	7.2	0.39	0.38	0.33	0.44
C ₃ grass	<i>Agropyron repens</i>	7.8	8.4	8.9	7.2	0.33	0.23	0.27	0.29
	<i>Bromus inermis</i>	6.8	8.1	7.6	7.3	0.29	0.33	0.31	0.31
	<i>Koeleria cristata</i>	6.1	6.3	6.7	5.6	0.22	0.19	0.26	0.14
	<i>Poa pratensis</i>	6.4	6.9	6.9	6.4	0.38	0.27	0.35	0.30
	Mean	6.8	7.4	7.5	6.6	0.29	0.25	0.29	0.26
Forb	<i>Achillea millefolium</i>	7.0	7.3	7.3	7.1	0.19	0.26	0.19	0.26
	<i>Anemone cylindrica</i>	7.6	8.0	7.4	8.2	0.75	0.71	0.37	1.09
	<i>Asclepias tuberosa</i>	7.7	7.8	7.8	7.8	1.84	1.04	1.27	1.61
	<i>Solidago rigida</i>	6.8	7.2	7.1	7.0	0.23	0.25	0.16	0.32
	Mean	7.3	7.6	7.4	7.5	0.77	0.56	0.51	0.82
Legume	<i>Amorpha canescens</i>	8.3	8.0	8.5	7.8	1.39	1.18	0.99	1.58
	<i>Lespedeza capitata</i>	7.8	7.6	8.0	7.4	1.51	0.94	1.11	1.34
	<i>Lupinus perennis</i>	8.7	8.6	8.7	8.5	1.67	2.11	1.57	2.21
	<i>Petalostemum villosum</i>	7.8	8.0	8.1	7.6	1.71	0.80	1.04	1.47
	Mean	8.1	8.1	8.4	7.9	1.57	1.26	1.18	1.65

percentage SW was plotted against fine root biomass, there was significantly higher percentage SW under elevated than ambient CO₂ and lower percentage SW under elevated than ambient N (Fig. 4a,b).

Soil solution N

The high N treatment increased soil solution nitrate and total N by roughly 40–50% on average (Table 7; Fig. 2). In contrast, and supporting H_{7a}, elevated CO₂ reduced soil nitrate and total N by roughly 20–25% (Table 7; Fig. 2), consistent with increased fine root biomass under elevated CO₂. Functional groups differed in soil solution N, with legumes having the highest and C₃ grasses the lowest levels (Tables 2, 7). These functional group differences were roughly opposite to patterns of fine root biomass. Functional groups differed in the extent of depletion of soil solution N due to CO₂, with the C₄ group having little effect compared to the C₃ groups in general (Tables 5, 7). For N treatment, legumes had a significantly greater increase in soil solution N than the C₃ grass species,

likely due to the degree of soil solution N depletion associated with differences in fine root biomass.

Accounting for variation in fine root biomass, there were significant differences in soil N concentrations due to N treatments, but not to CO₂. When data for all plots were pooled and soil solution N was plotted against fine root biomass, plots had significantly higher soil N under high than low N but did not differ under the contrasting CO₂ treatments (Fig. 4c,d).

For nonlegumes, there was an inverse relationship between total plant N pools and soil solution N concentrations ($P < 0.001$), likely because vegetation on plots with higher fine root biomass take up N (which is incorporated into plant tissues) while driving down the soil solution N concentration (Fig. 5). At any soil solution N pool, the N deposition treatment had higher plant N pools. Two of the legumes (*Petalostemum* and *Amorpha*) had slightly higher soil solution N pools at any given plant N pool but fit more or less within the general scatterplot relationship for the non-legumes ($P < 0.001$, $r^2 = 0.44$ for the 56 plots within each N treatment level) (Fig. 5). By contrast, the other two legumes, *Lespedeza*

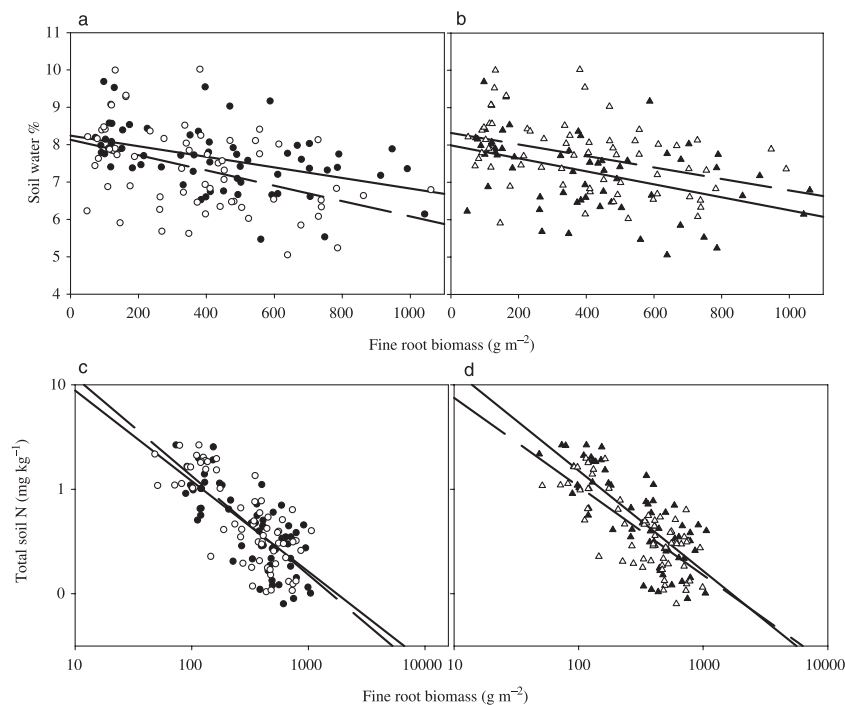


Fig. 4 (a,b) Mean percentage volumetric soil water (averaged over 18 sampling periods over two years) in relation to mean fine root biomass (averaged over 4 harvests in 2 yr) for 16 species monocultures under contrasting CO_2 and N treatments (pooled across the other treatment). Open circles, ambient CO_2 ; closed circles, elevated CO_2 ; open triangles, low N; closed triangles, high N. The slopes were not significantly different among treatments in either case, but the elevation of the line was significantly ($P < 0.025$) different in each case. (c,d) Mean soil solution N concentration (mg/kg) in relation to mean fine root biomass (both averaged over 4 harvests in 2 yr) for 16 species monocultures under contrasting CO_2 and N treatments (pooled across the other treatment). Open circles, ambient CO_2 ; closed circles, elevated CO_2 ; open triangles, low N; closed triangles, high N. The slopes were not significantly different among treatments in either case, and the elevation of the line was significantly ($P < 0.025$) different for contrasting N treatments but not contrasting CO_2 treatments.

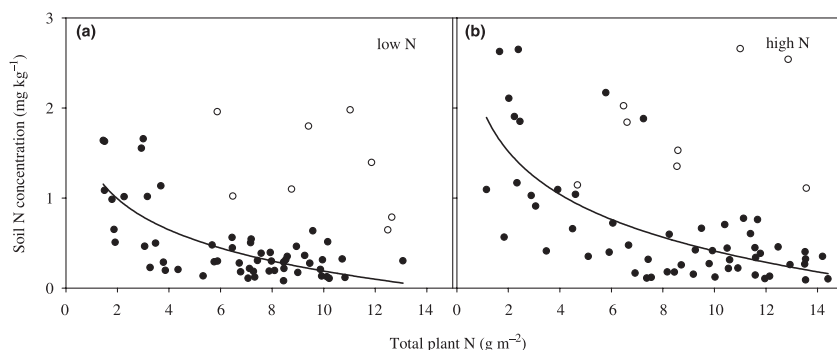


Fig. 5 Mean soil solution N concentration (mg/kg) in relation to mean total plant N (g/m^2), for plots under low and high N treatments, pooled across CO_2 treatments. The curves shown are for all nonN-fixers plus *Amorpha* and *Petalostemum*, and the relationship between \log_{10} soil N and \log_{10} plant N was significant ($P < 0.001$, $R^2 = 0.44$) in both cases. Closed circles, 14 species; open circles, *Lepedeza* and *Lupinus*.

and *Lupinus*, always had higher soil solution N pools at any given plant N pool and plots for these two species fell far from the relationship for the other 14 species (Fig. 5).

Discussion

A surprising number of species in this study had modest or negligible increases in biomass production in response to CO_2 or N fertilization (Fig. 1). Our major hypotheses regarding functional group responses to CO_2 and N were neither consistently supported nor rejected, and will be discussed below. Overall, these grassland species were less responsive to elevated CO_2 (see also Lee *et al.*, 2001) than has been generally found elsewhere, although some studies have found weak responses to elevated CO_2 (Koch & Mooney, 1996; Körner & Bazzaz, 1996; Curtis & Wang, 1998; Poorter, 1998). The mean increase due to elevated CO_2 in total biomass of all 12 C_3 species in our study was 16%, considerably less than

the 29%, 42% and 44% increases, respectively, reported in reviews of C_3 woody plants (Curtis & Wang, 1998), C_3 grasses in general (Poorter, 1993), and C_3 Poaceae (Wand *et al.*, 1999). The mean response of biomass to elevated CO_2 for the C_4 grasses in our study (-3%) is also less than the mean increases of 22% and 33% reported for C_4 grasses in general (Poorter, 1993) and for C_4 Poaceae (Wand *et al.*, 1999), respectively. Possible explanations for the limited CO_2 response in our study compared with previous studies include the relatively low fertility of our site, the general high level of adaptation to infertility among our study species, and the moist conditions during the study years. Alternatively, only a small fraction of the studies used in the reviews cited above were grown under realistic field conditions, and even fewer without chambers. It is important to note that a high degree of photosynthetic acclimation among these species (Lee *et al.*, 2001) can broadly explain the small growth response to elevated CO_2 at our site, though the correlation between individual species photosynthetic

vs. biomass responses was weak (P. B. Reich *et al.*, unpublished). Testing whether long-term responses to elevated CO₂ of grassland species in the field are generally less than those of shorter-term and more controlled experiments will require a larger data base than is currently available.

Functional group and species responses to elevated CO₂

As a group, C₄ grasses did show significantly less biomass enhancement in response to elevated CO₂ than the C₃ functional groups (supporting H₁) and no C₄ grass species showed a significant increase. These biomass responses are consistent with leaf level photosynthesis data; during the 2-yr study period, the C₄ grass species showed no enhancement of photosynthesis under elevated CO₂, in contrast to a modest increase on average for the C₃ species (Lee *et al.*, 2001).

Two of the four legumes in our study, *Lupinus* and *Lespedeza*, showed marked production increases under elevated CO₂ but the other two did not. Thus (H₂) – a hypothesis based on the general tendency of N-fixers to respond more to elevated CO₂ than nonfixers (Soussana & Hartwig, 1996; Clark *et al.*, 1997; Hebeisen *et al.*, 1997; Lüscher *et al.*, 1997, 1998; Schenk *et al.*, 1997) – apparently did not hold. Can we explain this marked difference in response among legume species?

Although we know little about variation in the degree of N-fixation among these four legumes, we can make some indirect inferences based on the data from this study. *Lupinus* and *Lespedeza* plots had somewhat higher soil solution N pools and two to three times as much plant N per plot as *Amorpha* and *Petalostemum*, which we take as evidence of greater N fixation in the former two species, since all else being equal, with greater root biomass they should have otherwise depleted the soil solution N to a level lower than the other two legumes. Moreover, for all nonfixers plus *Amorpha* and *Petalostemum* pooled, there was a significant inverse relationship (Fig. 5) between soil solution N and plant N pools, consistent with earlier studies relating differences among species in root biomass to depletion of soil solution N pools in grasslands at Cedar Creek (Tilman & Wedin, 1991). *Lupinus* and *Lespedeza* did not follow this inverse relationship and had much higher soil solution N for a given plant N pool, suggesting relatively high rates of N fixation.

In addition, for the 12 nonfixer species (plus *Amorpha* and *Petalostemum*) the plant N pool was relatively unchanged under elevated CO₂. This suggests that increased biomass in response to elevated CO₂ by itself did not result in greater total plant N acquisition in nonfixers. In contrast, *Lupinus* and *Lespedeza* had marked increases in plant N pool in elevated CO₂ (4.0 and 3.3 g m⁻², respectively). Hence, it is plausible that *Lupinus* and *Lespedeza* more vigorously fix N than the other legumes, and responded to elevated CO₂ by increasing their own N supply, leading to heightened uptake of both C and N. In support of this idea, using the ¹⁵N isotope dilution method for *Lupinus* monocultures, the proportion of

N derived from fixation increased by 38% under elevated CO₂ (T. D. Lee *et al.*, unpublished). Thus, based on indirect evidence from this study, the legumes with greater N-fixation tendencies were more responsive to elevated CO₂ than those with lesser N-fixation tendencies, supporting the concept of N-fixation enhancing CO₂ responses (H₂), but suggesting that heterogeneity among N-fixers may limit the generalizability of this idea.

Functional group and species responses to increased N supply

Our hypotheses about biomass responses to increased N supply (H₃ to H₅) were also supported with mixed results. The C₃ grasses increased biomass the most at high N and the legumes as a group responded little to N addition, as predicted (H₃, H₄). However, one of the four legume species, *Petalostemum*, did increase biomass at high N, and perhaps related, this species did not respond positively to elevated CO₂ and may have had a low rate of N fixation. All eight grasses, regardless of photosynthetic pathway, had large increases in aboveground biomass at high N, and 7 of the 8 also had large increases belowground. However, surprisingly, only one of four forbs responded positively to N addition in terms of total biomass. Perhaps these species are poor competitors for soil N in relationship to microbes? If that was so (i.e. microbial uptake depleted the available soil N), soil solution N should be low, yet it was higher for the forbs than for the grasses. Moreover, two of the three forbs which did not increase biomass at high N did have higher tissue percentage N. The failure of these species to respond positively with increased production under high N is difficult to explain.

Finally, there were no CO₂ × N interactions and hence no tendency for any functional group to respond differently to CO₂ as a function of N supply (H₅). Elevated N supply did not increase monoculture response to CO₂, unlike a number of other studies (e.g. Owensby *et al.*, 1994; Zanetti *et al.*, 1996; Curtis & Wang, 1998; Zak *et al.*, 2000). Whether this is due to the relatively smaller N addition in this study (4 g N m⁻² yr⁻¹) than in some others (e.g. N treatments were as high as 56 g N m⁻² yr⁻¹ and more closely mimic agricultural N addition rates, Zanetti *et al.*, 1996) can not be answered without a larger number of studies of elevated CO₂ effects under different N addition regimes.

Controls on percentage soil water

How can we explain CO₂ and N treatment effects on percentage soil water? Biomass per plot had a large influence on percentage SW (Fig. 4), and both species and treatments contributed to variation in biomass, although the former was dominant. For N, even for a given fine root mass, plots under high N had lower percentage SW. There was no effect of N on leaf diffusive conductance (Lee *et al.*, 2001) (and hence on leaf-level water loss), but high N did lead to a lower root

fraction (Tables 3, 5). Hence, high N plots support a higher aboveground biomass and likely a higher leaf area index and transpirational surface, perhaps explaining the lower percentage SW under high N at a given fine root biomass.

By contrast, elevated CO₂ leads to greater biomass which, all else being equal, should lead to greater depletion of soil water. However, individual species actually had lower percentage SW under elevated CO₂ despite greater biomass (Table 7) and overall, percentage SW at any given fine root biomass was higher under elevated CO₂. Elevated CO₂ did not affect root fraction, so a shift in absorbing (roots) vs transpirational surfaces (canopy) can not be invoked as an explanatory factor. In a companion study (Lee *et al.*, 2001) we found a consistent and roughly 25% decrease in leaf conductance across species, functional groups and years. An analysis (data not shown) that includes functional group and leaf conductance found both factors significantly ($P < 0.05$) associated with percentage SW, with percentage SW negatively related to conductance. Hence, reduced conductance contributes to the tendency for elevated CO₂ plots to have lower percentage SW.

Other functional traits and groupings

Variation in a number of other functional traits, including intrinsic growth rate, leaf gas exchange capacity, resource depletion capacity, sink strength, plant strategy, and root symbiont status, has been proposed to explain differential responsiveness to elevated CO₂, added N and a variety of other global change factors (Reich, 1987; Hunt *et al.*, 1991, 1993; Díaz, 1995; Poorter *et al.*, 1996; Wedin & Tilman, 1996). Such explanations have met with some success with other factors, but less so vis-à-vis CO₂ (Poorter *et al.*, 1996; Volin & Reich, 1996). None of these hypotheses moreover, appear capable of fully explaining differences in responses among the 16 species in this study. The species that were most productive under ambient CO₂ and soil N conditions were not consistently responsive to either elevated CO₂ or N, nor were the less productive species consistently responsive. Similarly, for 10 of the 16 species we also measured relative growth rate of seedlings under controlled conditions (P. B. Reich *et al.*, unpublished) and found no relationship between proportional biomass enhancement due to CO₂ in field plots and individual seedling RGR. Species which reduce the soil N concentration under ambient conditions did generally respond more positively to N enrichment, but with two important exceptions (*Achillea* and *Solidago*).

Among the nonlegumes, tissue percentage N in the low N treatment did not predict which species would respond to N enrichment (data not shown), but it did predict the relative responsiveness to elevated CO₂ (Fig. 6). For C₃ and C₄ species separately, species with greater aboveground tissue percentage N had a greater increase in biomass in response to elevated CO₂ than those with lower percentage N. The slopes were similar for both groups, and the relationship was at a lower

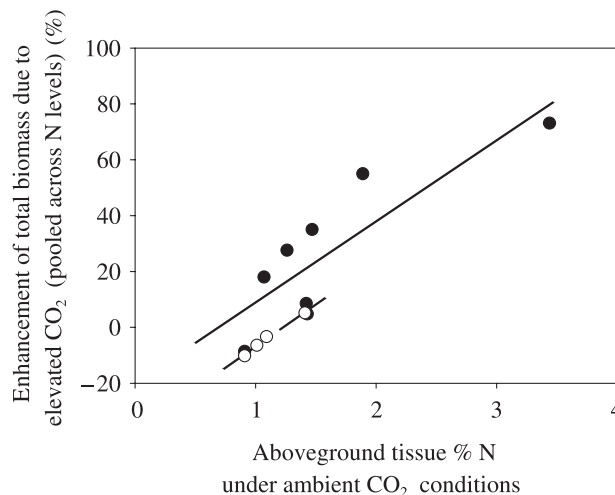


Fig. 6 The percentage enhancement of total biomass due to elevated CO₂ for all nonN-fixer species, in relation to the aboveground tissue percentage N of plants under ambient CO₂ conditions. Open circles, C₄ species; closed circles, C₃ species. The regression relationships were significant ($P < 0.001$) for C₃ and C₄ species considered separately ($R^2 = 0.72$ and 0.99 , respectively), with a similar slope ($P > 0.10$) but different intercept ($P < 0.05$).

‘elevation’ (i.e. intercept was lower) for the C₄ than C₃ species. Why do these patterns occur? A plausible hypothesis follows from physiological principles. Species with higher tissue percentage N have a higher carboxylation capacity (Evans, 1989) such that if they were ‘operating’ on the steep part of the A-C_i curve (Farquhar & Sharkey, 1982) they should have a greater enhancement due to elevated CO₂ than species with lower tissue percentage N (this could explain patterns within each group). Moreover, since C₄ plants are already near saturated at ambient CO₂ (i.e. on a shallower part of the curve) they are less responsive as a group to elevated CO₂, even for a given leaf percentage N (this could explain the lower position of the overall line for this group). With their different C : N dynamics, legumes would not necessarily be expected to follow similar patterns. Although many processes beyond leaf level photosynthesis influence ecosystem scale biomass enhancement to elevated CO₂ (such as canopy architecture, turnover rates, tissue morphology, biomass distribution, and phenology), leaf-level processes provide the starting point for carbon acquisition which leads to biomass accumulation, and which drives increased biomass accumulation under elevated CO₂. Whether this is a robust general relationship requires further testing in other common garden experiments in the field.

Implications and conclusions

There have been many isolated potted plant studies of elevated CO₂ responses. Studies of monocultures in the field with free-air CO₂ enrichment provide information that is closer to natural conditions. Moreover, if we hope to be able to use functional groupings to generalize about, and quantitatively model responses

to elevated CO₂ and N of mixed species communities, then we need to understand variation in species and functional group responses under simpler monoculture conditions. If responses to elevated CO₂ and N deposition are largely a function of changing resource supply then growth in mixed communities is complicated by species interactions that can change as a result of these agents (Owensby *et al.*, 1993, 1994). Competition often involves growing with other species that differentially utilize resources (e.g. soil solution N, Tilman & Wedin, 1991) or supply them (e.g. N-fixers) by dint of species ecophysiological differences. Hence, understanding species responses to enriched CO₂ or N in mixed communities is complicated because competition ensures that any given species does not necessarily have access to increased supplies of a resource that is added to an ecosystem. Nonetheless, responses of species mixtures to elevated CO₂ may be related to their responses in monocultures (Navas *et al.*, 1999). Therefore, interpretation and evaluation of species under interspecific competition (e.g. Warwick *et al.*, 1998; Leadley *et al.*, 1999) will be aided by a better understanding of their responses growing under intraspecific competition, as in the monocultures of this study.

Our results are only somewhat encouraging vis-à-vis the use of functional groups. Functional groups did often respond significantly differently to CO₂ or N, and hence do provide some meaningful information without knowledge about individual species. However, there was also substantial variation in response among species within groups. Alternative classifications based on measured continuous traits were generally no more useful than the *a priori* defined functional groups. These results suggest that current trait-based functional classifications may be useful, but not sufficient for understanding plant and ecosystem responses to elevated CO₂ and N deposition.

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