Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO_2 and N availability regimes? A field test with 16 grassland species

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Summary

• To evaluate whether functional groups have a similar response to global change, the responses to CO_2 concentration and N availability of grassland species from several functional groups are reported here.

• Sixteen perennial grassland species from four trait-based functional groups (C_3 grasses, C_4 grasses, non-leguminous forbs, legumes) were grown in field monocultures under ambient or elevated (560 μmol mol^{-1}) CO_2 using free-air CO_2 enrichment (FACE), in low N (unamended field soil) or high N (field soil +4 g N m^{-2} $years^{-1}$) treatments.

• There were no $CO_2 \times N$ interactions. Functional groups responded differently to CO_2 and N in terms of biomass, tissue N concentration and soil solution N. Under elevated CO_2, forbs, legumes and C_3 grasses increased total biomass by 31%, 18%, and 9%, respectively, whereas biomass was reduced in C_4-grass monocultures. Two of the four legume species increased biomass and total plant N pools under elevated CO_2, probably due to stimulated N-fixation. Only one species markedly shifted the proportional distribution of below- vs aboveground biomass in response to CO_2 or N.

• Although functional groups varied in responses to CO_2 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 CO_2 and N availability.

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

Introduction

Generalizing about responses of different plant species to elevated atmospheric CO_2 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 CO_2 and N enrichment.

1) Intrinsic differences in photosynthetic biochemistry should lead to markedly greater responsiveness to elevated CO_2 for C_3 than C_4 plants (H_1). Although supported by

theory and early field studies (Pearcy & 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 CO_2 more than in nonfixers (H_2), because the former should be less N-limited. It has long been known that elevated CO_2 stimulates legume growth and N_2 fixation (Finn & Brun, 1982; Zanetti *et al.*, 1996). N-fixing species have often shown a stronger biomass response to elevated CO_2 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 CO_2 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 (H_3) that C_3 grasses considered more disturbance adapted and nitrophilic, should respond more to increase in N supply than C_4 grasses (cf Wedin & Tilman, 1996). Moreover, being less N-limited, the legumes should also be less responsive to N addition than the non-fixers (H_4).

5) In addition to understanding the effects of elevated CO_2 or N singularly, given the potential for the CO_2 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 CO_2 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 CO_2 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 CO_2 than legumes (see H_2) and be more likely to show a $CO_2 \times N$ interaction (H_5).

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 CO_2 (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 CO_2 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 CO_2 and N supply both lead to increased biomass, we hypothesize (H_7) 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 CO_2 (H_{7a}). Under elevated CO_2, 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 $CO_2 \times N$ 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 CO_2 and N deposition (Reich *et al.*, 2001). In particular, we assessed whether functional groups, growing in monoculture plots in a free-air CO_2 enrichment (FACE) experiment, differed in their acquisition and use of C, N and water in response to combined treatment with elevated CO_2 and N deposition.

Materials and Methods

The BioCON (Biodiversity, CO_2 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 CO_2 rings, a free-air CO_2 enrichment (FACE) system (Lewin *et al.*, 1994) was used during the 1998 and 1999 growing seasons to maintain the CO_2 concentration at 560 μmol mol^{-1}. Three ambient rings (368 μmol mol^{-1} CO_2) were treated identically but without additional CO_2. The experimental treatments were arranged in complete factorial combination of CO_2 (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. CO_2 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. CO_2 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 CO_2 on dark respiration (Tjoelker *et al.*, 2001). During CO_2 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 C_4 grasses (*Andropogon gerardii* Vitman, *Bouteloua gracilis*, *Schizachyrium scoparium* (Michaux) Nash, *Sorghastrum nutans* (L.)(Nash), four C_3 grasses (*Agropyron repens* (L.) Beauv., *Bromus inermis* Leysser, *Koeleria cristata* Pers, *Poa pratensis* L.), four N-fixing legumes (*Amorpha canescens* Pursh, *Lespedeza 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 CO_2 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 CO_2 (1 df) was tested against the random effect of ring nested within CO_2 (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 CO_2, 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. C_3 vs C_4), 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 CO_2 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 C_3 grass monocultures highest and legumes lowest. For total biomass, averaged across all species or functional groups, there were marginally significant main effects of CO_2 (+11%, mean of enhancement) and N (+7%) (Tables 2, 3; Fig. 1) and there were no significant $CO_2 \times N$ interactions (Tables 2, 3). In fact, there were no significant $CO_2 \times N$ interactions, or significant three–way interactions involving CO_2 and N, for any of the measured variables in this study (refuting H_5), and hence responses to CO_2 and N will be presented separately, pooled across levels of the other variable.

Biomass response to elevated CO_2

There were marked differences among functional groups in terms of biomass response to CO_2 (Tables 1, 2; Figs 1, 2). Forbs, legumes, and C_3 grasses increased total biomass by 31%, 18%, and 9%, respectively, under elevated CO_2 whereas C_4 grass monocultures had 3% lower total biomass. The interaction term was significant ($P < 0.10$) for the CO_2–group interaction and more so ($P < 0.002$) for the C_3 vs C_4 contrast (Table 2), indicating that the C_4 grasses did have less enhancement of biomass than C_3 species in general, supporting (H_1). There was no evidence that the N-fixing legumes responded more positively to elevated CO_2 than the nonfixing C_3 species (Figs 1, 2; Tables 1, 2), refuting (H_2). The effects of CO_2 on biomass, and differences in response among functional groups, were largely manifest belowground (Tables 1, 2).

There was substantial variation in response to elevated CO_2 among species, shown by significant species–CO_2 interactions

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Table 1 Means of belowground and aboveground biomass of species under contrasting CO_2 and N treatments for 128 plots. Values shown are for 4 plots per species at each CO_2 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

		Belowground biomass (g m^{-2})				Aboveground biomass (g m^{-2})			
Functional group	Species	Ambient CO_2	Elevated CO_2	Low N	High N	Ambient CO_2	Elevated CO_2	Low N	High N
C_4 grass	*Andropiogon gerardii*	575	525	543	557	261	283	263	282
	Bouteloua gracilis	472	562	471	564	253	200	209	244
	Schizachyrium scoparius	359	349	293	415	177	153	133	196
	Sorghastrum nutans	569	513	614	468	248	221	199	270
	Mean	493	489	480	502	236	215	204	247
C_3 grass	*Agropyron repens*	806	1114	883	1037	308	307	282	333
	Bromus inermis	828	755	750	834	306	281	253	333
	Koeleria cristata	645	714	666	692	253	225	194	284
	Poa pratensis	991	1058	929	1120	168	200	123	245
	Mean	817	910	807	920	256	252	210	299
Forb	*Achillea millefolium*	700	1014	944	770	268	293	266	295
	Anemone cylindrica	183	288	324	147	36	53	60	29
	Asclepias tuberosa	97	172	91	179	11	14	12	13
	Solidago rigida	597	690	706	581	315	386	328	372
	Mean	395	541	516	419	160	185	167	179
Legume	*Amorpha canescens*	172	160	208	124	59	26	59	26
	Lespedeza capitata	274	407	315	366	67	153	143	77
	Lupinus perennis	216	303	270	250	279	383	339	323
	Petalostemum villosum	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

	$CO_2 \times N \times$ Functional group analyses								
Parameter	R^2	CO_2	N	Group	$CO_2 \times$ Group	$CO_2 \times$ (C_3 vs C_4)	$CO_2 \times C_3$ N-fixer vs C_3 nonfixer	N x 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

R^2 shown for whole model. *P*-values shown for main effects and interactions (bolded when $P < 0.10$). Interactions involving $CO_2 \times N$ 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.

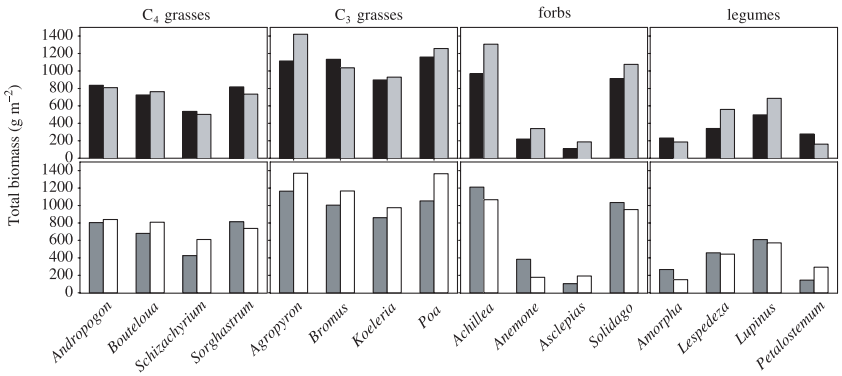

Fig. 1 Total biomass (aboveground plus belowground, 0–20 cm depth) (g m^{-2}) of 16 species as affected by elevated vs ambient CO_2 treatments (pooled across N treatments) and by high N (addition of 4 g N m^{-2} yr^{-1}) vs unamended soil treatments (pooled across CO_2 treatments). Top panel: grey columns, 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

	$CO_2 \times N \times$ Species analyses					
Parameter	R^2	CO_2	N	Species	CO_2 x Species	N x 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 $CO_2 \times N$ 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 g m^{-2}, +35%), a C_3 grass, *Agropyron* (+307 g m^{-2}, +28%) and two legumes, *Lespedeza* (+218 g m^{-2}, +64%) and *Lupinus* (+190 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 g m^{-2}, +30%), *Agropyron* (+206 g m^{-2}, +18%) and *Schizachyrium* (+163 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%

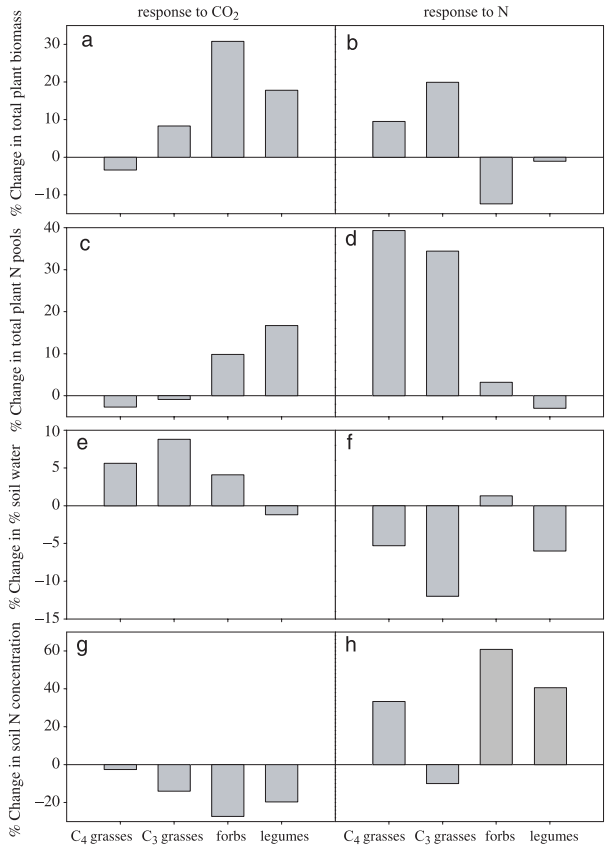

Fig. 2 Proportional response of total biomass, plant N pool, percentage soil water, and soil solution N concentration to CO_2 and N treatments (otherwise pooled) on average for species in four functional groups. See Table 2 for statistics.

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 × N or group–CO_2 interactions for root fraction (Table 2), but there were significant species × 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

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

	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 5 Means of root fraction (root biomass/total biomass) of species under contrasting CO_2 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	*Andropogon gerardii*	0.69	0.65	0.67	0.67
	Bouteloua gracilis	0.65	0.74	0.69	0.69
	Schizachyrium scoparius	0.68	0.69	0.70	0.68
	Sorghastrum nutans	0.69	0.69	0.75	0.63
	Mean	0.68	0.69	0.70	0.67
C_3 grass	*Agropyron repens*	0.73	0.79	0.76	0.75
	Bromus inermis	0.74	0.73	0.76	0.71
	Koeleria cristata	0.72	0.76	0.77	0.71
	Poa pratensis	0.86	0.84	0.89	0.82
	Mean	0.76	0.78	0.80	0.75
Forb	*Achillea millefolium*	0.71	0.77	0.77	0.72
	Anemone cylindrica	0.84	0.85	0.83	0.86
	Asclepias tuberosa	0.91	0.93	0.91	0.92
	Solidago rigida	0.64	0.64	0.68	0.61
	Mean	0.77	0.80	0.80	0.78
Legume	*Amorpha canescens*	0.81	0.85	0.82	0.83
	Lespedeza capitata	0.80	0.73	0.72	0.81
	Lupinus perennis	0.43	0.43	0.43	0.43
	Petalostemum villosum	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 CO_2 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.

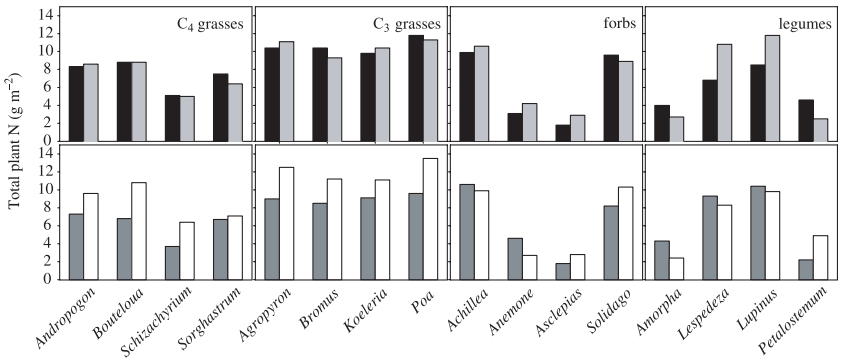

Fig. 3 Means of total plant N pools (g m^{-2}) of 16 species under contrasting CO_2 and N treatments. Top panel: grey columns, elevated CO_2; black columns, ambient CO_2. Bottom panel: grey columns, low N; white columns, high N. The mean standard errors of the adjusted LSMs were 0.8 g m^{-2} for species. 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, CO_2 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 C_4 grasses did not have lower percentage N under elevated CO_2 (Table 6). All C_3 functional groups tended to have substantially lower tissue percentage N under elevated CO_2 (Table 6), but species varied markedly in this respect.

Table 6 Means of tissue percentage N of species under contrasting CO_2 and N treatments for 128 plots. Values shown are for 4 plots per species at each CO_2 or N level, pooled across treatments otherwise and averaged over August harvests in 1998 and 1999

		Aboveground % N				Belowground % N				Total % N			
Functional group	Species	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	*Andropogon gerardi*	1.09	1.15	0.93	1.31	0.95	1.00	0.88	1.07	0.98	1.05	0.90	1.13
	Bouteloua gracilis	1.41	1.33	1.11	1.63	1.14	1.07	0.94	1.26	1.24	1.16	1.01	1.40
	Schizachyrium scoparius	1.01	1.20	1.04	1.17	0.89	0.90	0.82	0.98	0.94	1.01	0.91	1.05
	Sorghastrum nutans	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	*Agropyron repens*	1.26	0.89	0.96	1.19	0.84	0.72	0.72	0.84	0.94	0.73	0.75	0.92
	Bromus inermis	0.91	1.00	0.80	1.10	0.91	0.85	0.87	0.89	0.91	0.89	0.85	0.94
	Koeleria cristata	1.43	1.30	1.20	1.53	0.95	1.03	1.01	0.97	1.12	1.12	1.06	1.18
	Poa pratensis	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	*Achillea millefolium*	1.47	1.27	1.35	1.39	0.85	0.71	0.78	0.78	1.06	0.87	0.93	1.00
	Anemone cylindrica	1.89	1.55	1.57	1.88	1.37	1.20	1.19	1.38	1.49	1.30	1.27	1.51
	Asclepias tuberosa	3.44	2.94	3.51	2.86	1.45	1.42	1.49	1.38	1.90	1.65	1.77	1.78
	Solidago rigida	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	*Amorpha canescens*	2.70	2.17	2.47	2.40	1.48	1.44	1.40	1.53	1.73	1.55	1.58	1.71
	Lespedeza capitata	2.35	1.95	2.12	2.18	1.89	1.92	1.98	1.83	2.00	1.93	2.02	1.91
	Lupinus perennis	1.52	1.43	1.43	1.52	2.15	2.12	2.29	1.99	1.87	1.78	1.88	1.78
	Petalostemum villosum	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 percentageN for species and 0.14, 0.07, and 0.07% for aboveground, belowground and total percentageN for functional groups.

Agropyron, *Amorpha*, *Anemone*, *Asclepias*, *Lespedeza*, and *Poa* (equally representing the three C_3 groups) decreased percentage N by the greatest amounts under elevated CO_2, and most other C_3 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 (H_7) (Tables 2, 3, 7; Fig. 2). Moreover, species and functional groups differed marginally in percentage SW and in how percentage SW changed with CO_2 and N. C_3 grasses had the lowest percentage SW, C_4 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 C_3 grass) had the lowest percentage SW and *Lupinus*, *Agropyron* and several other species the highest.

On average, C_3 grasses showed the greatest increase in percentage SW with elevated CO_2 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 CO_2 (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 CO_2, consistent with its slightly lower biomass under elevated CO_2. For N treatments, *Agropyron* and *Koeleria* (C_3 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 CO_2 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 CO_2 and N treatments for 128 plots. Values shown are for 4 plots per species at each CO_2 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

		% soil water				Soil solution N conc. (mg/kg)			
Functional group	Species	Amb CO_2	Elev CO_2	Low N	High N	Amb CO_2	Elev CO_2	Low N	High N
C_4 grass	*Andropogon gerardii*	7.4	7.5	7.7	7.2	0.42	0.38	0.33	0.47
	Bouteloua gracilis	6.5	7.3	7.1	6.8	0.55	0.45	0.39	0.61
	Schizachyrium scoparius	7.6	7.9	7.8	7.6	0.38	0.41	0.31	0.48
	Sorghastrum nutans	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	*Agropyron repens*	7.8	8.4	8.9	7.2	0.33	0.23	0.27	0.29
	Bromus inermis	6.8	8.1	7.6	7.3	0.29	0.33	0.31	0.31
	Koeleria cristata	6.1	6.3	6.7	5.6	0.22	0.19	0.26	0.14
	Poa pratensis	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	*Achillea millefolium*	7.0	7.3	7.3	7.1	0.19	0.26	0.19	0.26
	Anemone cylindrica	7.6	8.0	7.4	8.2	0.75	0.71	0.37	1.09
	Asclepias tuberosa	7.7	7.8	7.8	7.8	1.84	1.04	1.27	1.61
	Solidago rigida	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	*Amorpha canescens*	8.3	8.0	8.5	7.8	1.39	1.18	0.99	1.58
	Lespedeza capitata	7.8	7.6	8.0	7.4	1.51	0.94	1.11	1.34
	Lupinus perennis	8.7	8.6	8.7	8.5	1.67	2.11	1.57	2.21
	Petalostemum villosum	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 CO_2 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 CO_2 reduced soil nitrate and total N by roughly 20–25% (Table 7; Fig. 2), consistent with increased fine root biomass under elevated CO_2. Functional groups differed in soil solution N, with legumes having the highest and C_3 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 CO_2, with the C_4 group having little effect compared to the C_3 groups in general (Tables 5, 7). For N treatment, legumes had a significantly greater increase in soil solution N than the C_3 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 CO_2. 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 CO_2 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*

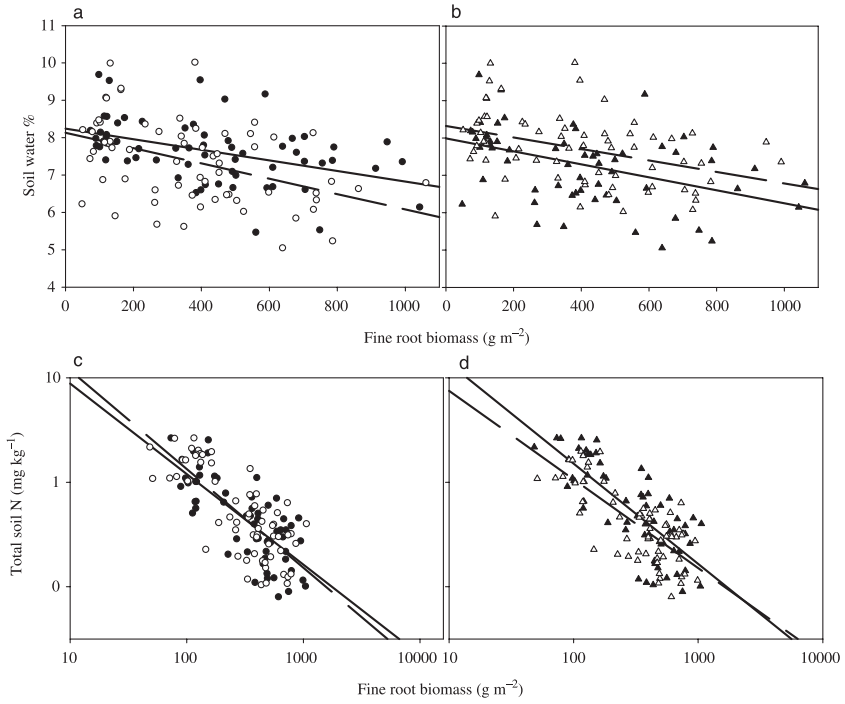

Fig. 4 (a,b) Mean percentage volumetric soil water (averaged over 18 sampling periods over two years) in relation to mean fine root biomass (averaged over 4 harvests in 2 yr) for 16 species monocultures under contrasting CO_2 and N treatments (pooled across the other treatment). Open circles, ambient 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.

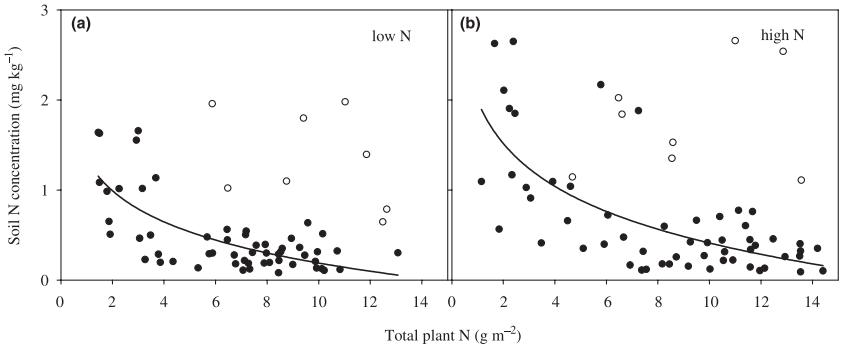

Fig. 5 Mean soil solution N concentration (mg/kg) in relation to mean total plant N (g m^{-2}), for plots under low and high N treatments, pooled across CO_2 treatments. The curves shown are for all nonN-fixers plus *Amorpha* and *Petalostemeum*, and the relationship between $\log_{10}$ soil N and $\log_{10}$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

As a group, C_4 grasses did show significantly less biomass enhancement in response to elevated CO_2 than the C_3 functional groups (supporting H_1) and no C_4 grass species showed a significant increase. These biomass responses are consistent with leaf level photosynthesis data; during the 2-yr study period, the C_4 grass species showed no enhancement of photosynthesis under elevated CO_2, in contrast to a modest increase on average for the C_3 species (Lee *et al.*, 2001).

Two of the four legumes in our study, *Lupinus* and *Lespedeza*, showed marked production increases under elevated CO_2 but the other two did not. Thus (H_2) – a hypothesis based on the general tendency of N-fixers to respond more to elevated CO_2 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 CO_2. This suggests that increased biomass in response to elevated CO_2 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 CO_2 (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 CO_2 by increasing their own N supply, leading to heightened uptake of both C and N. In support of this idea, using the ^{15}N isotope dilution method for *Lupinus* monocultures, the proportion of N derived from fixation increased by 38% under elevated CO_2 (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 CO_2 than those with lesser N-fixation tendencies, supporting the concept of N-fixation enhancing CO_2 responses (H_2), 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 (H_3 to H_5) were also supported with mixed results. The C_3 grasses increased biomass the most at high N and the legumes as a group responded little to N addition, as predicted (H_3, H_4). 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 CO_2 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 $CO_2 \times N$ interactions and hence no tendency for any functional group to respond differently to CO_2 as a function of N supply (H_5). Elevated N supply did not increase monoculture response to CO_2, 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 CO_2 effects under different N addition regimes.

Controls on percentage soil water

How can we explain CO_2 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 CO_2 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 CO_2 despite greater biomass (Table 7) and overall, percentage SW at any given fine root biomass was higher under elevated CO_2. Elevated CO_2 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 CO_2 plots to have lower percentage SW.

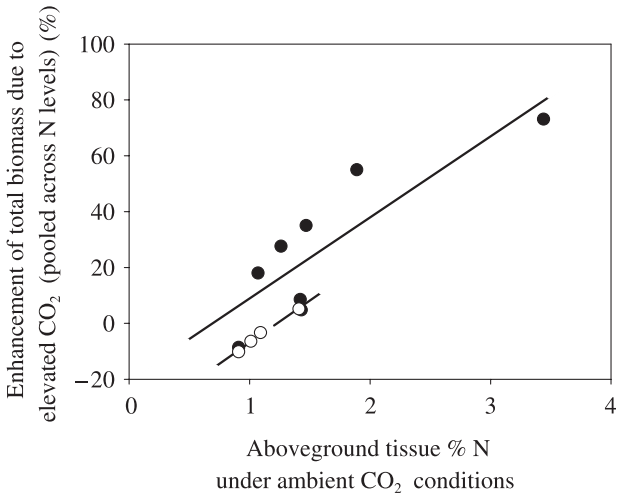

Fig. 6 The percentage enhancement of total biomass due to elevated CO_2 for all nonN-fixer species, in relation to the aboveground tissue percentage N of plants under ambient CO_2 conditions. Open circles, C_4 species; closed circles, C_3 species. The regression relationships were significant ($P < 0.001$) for C_3 and C_4 species considered separately ($R^2 = 0.72$ and 0.99, respectively), with a similar slope ($P > 0.10$) but different intercept ($P < 0.05$).

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 CO_2, 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 CO_2 (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 CO_2 and soil N conditions were not consistently responsive to either elevated CO_2 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 CO_2 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 CO_2 (Fig. 6). For C_3 and C_4 species separately, species with greater aboveground tissue percentage N had a greater increase in biomass in response to elevated CO_2 than those with lower percentage N. The slopes were similar for both groups, and the relationship was at a lower 'elevation' (i.e. intercept was lower) for the C_4 than C_3 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 CO_2 than species with lower tissue percentage N (this could explain patterns within each group). Moreover, since C_4 plants are already near saturated at ambient CO_2 (i.e. on a shallower part of the curve) they are less responsive as a group to elevated CO_2, 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 CO_2 (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 CO_2. 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 CO_2 responses. Studies of monocultures in the field with free-air CO_2 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 CO_2 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 CO_2 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 CO_2 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 CO_2 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 CO_2 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 CO_2 and N deposition.

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