Leaf gas exchange responses of 13 prairie grassland species to elevated CO_2 and increased nitrogen supply

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Summary

• Leaf gas exchange responses to elevated CO_2 and N are presented for 13 perennial species, representing four functional groups: C_3 grasses, C_4 grasses, legumes, and nonleguminous forbs. Understanding how CO_2 and N effects interact is important to predict plant community response to global change.

• Plants were field-grown in monoculture under current ambient and elevated (560 μmol mol^{-1}) CO_2 concentrations (free-air CO_2 enrichment), in combination with soil N treatments, for two growing seasons.

• All species, regardless of functional group, showed pronounced photosynthetic acclimation to elevated CO_2, resulting in minimal stimulation of photosynthesis (A) averaging +15% in C_3 grasses, +8% in forbs, +7% in legumes and –2% in C_4 grasses. The effects of CO_2 and soil N supply did not interact for any leaf traits measured. Elevated CO_2 consistently decreased stomatal conductance (g_s) leading to 40% increase in A/g_s.

• This substantial acclimation of photosynthesis was greater in magnitude than in most field studies, and was associated with the combined effects of decreased g_s and decreased leaf N concentrations in response to growth under elevated CO_2.

Key words: elevated CO_2, nitrogen availability, photosynthetic acclimation, functional groups, prairie grassland, stomatal conductance.

Introduction

Rising atmospheric carbon dioxide (CO_2) concentration is predicted to have profound effects on ecosystems. Human activity is also altering the global nitrogen (N) cycle by substantially increasing the inputs of fixed forms of N, primarily by the extensive use of chemical fertilizers and combustion of fossil fuels (Vitousek *et al.*, 1997). The effects of elevated atmospheric CO_2 and increased soil N on vegetation likely interact in complex ways and differently at different scales. Although larger-scale field experiments are increasing in number and longevity, there continues to be a need for field experiments to determine the extent to which results found in controlled environment studies apply to intact plant communities (Polley, 1997).

Plant responses to elevated CO_2 are fundamentally mediated by photosynthesis (Drake *et al.*, 1997), and can potentially lead to a suite of morphological and growth changes. It is well documented that increased CO_2 enhances the photosynthetic rate and growth of most C_3 plants (Bowes, 1993). However, as larger-scale, longer-term studies are being conducted, findings indicate the degree of this response to be variable and its persistence over the long-term questionable. To date, photosynthetic responses to experimentally doubled CO_2 levels have ranged from neutral, even negative, to strongly positive in most crop systems where typical increases average from 20 to 40% (Schimel, 1995). Responses of less studied wild species in natural systems are often considerably lower in magnitude than crops and in some cases under protracted exposure to elevated CO_2, photosynthetic rates decline, resulting in a complete lack of enhancement (Bowes, 1993; Wand *et al.*, 1999). A reduction in photosynthetic capacity with exposure to elevated CO_2 may occur (e.g. photosynthetic acclimation; Gunderson & Wullschleger, 1994), in connection with changes in leaf chemistry and structure, as well as feedbacks governed by whole plant growth dynamics. Common responses to CO_2 enrichment include: decreases in the amount or activity of Rubisco, increases in total nonstructural carbohydrate concentrations, and decreases in leaf N concentration and leaf area to mass ratios (Curtis, 1996), which collectively should

lead to decreased rates of photosynthesis (Reich *et al.*, 1997). Understanding acclimation of photosynthesis to increased atmospheric CO_2 concentration, and how soil N availability affects this response, is critical for predicting plant community responses to environmental change.

Potential limitations of resources such as nutrients and light may mitigate photosynthetic responses to elevated CO_2 (Drake *et al.*, 1997). Considering the dynamics and influence of N on photosynthesis and growth becomes critical in the attempt to characterize individual plant, as well as ecosystem, responses to elevated CO_2 (Vitousek *et al.*, 1997). Since biologically available N currently limits productivity in most ecosystems, and because tissue N is a major determinant of photosynthesis (Nijs *et al.*, 1995; Reich *et al.*, 1997), low N may limit potential photosynthetic enhancement under elevated CO_2. Several simulation models predict that plant CO_2 responses are constrained by N limitation (McMurtie & Comins, 1996; Rastetter *et al.*, 1997), although actual evidence is mixed (Poorter, 1998), ranging from no consistent effect of nutrient availability on plant responsiveness to elevated CO_2 (Idso & Idso, 1994; Lloyd & Farquhar, 1996) to a decreased CO_2 sensitivity which is linked to low nutrient availability (Larigauderie *et al.*, 1988; Bazzaz, 1990; Oechel *et al.*, 1994; Leadley & Körner, 1996; Poorter, 1998). Thus, a plant's response to limiting factors other than atmospheric CO_2 may have a great impact on how it responds to elevated CO_2 (Bowes, 1993).

Research on differential responses to CO_2 among plant functional groups may help explain the large degree of inter-specific variation in acclimation to elevated CO_2. As a way to model the response of complex ecosystems based on group rather than species parameterizations, functional groups have been proposed because they have been found to help explain variations in species responses to the environment (Díaz, 1995). However, Díaz (1995) points out that plant responsiveness to elevated CO_2 may involve traits not usually considered in functional group definitions. Evaluating species in more natural field settings provides the opportunity to identify general patterns associated with strong or weak responses to elevated CO_2 and to test the usefulness of these functional classifications.

The overall objective of this experiment was to investigate how elevated CO_2 concentrations and increased soil N interact to affect leaf-level physiological processes of a variety of wild perennial plant species in a field setting and to examine if these responses to elevated CO_2 and N remain similar across two growing seasons. Our evaluation was done at the leaf level in order to identify potential physiological mechanisms underlying ecosystem response to these global change elements. In this study, the following questions are addressed: to what extent do field grown prairie species acclimate leaf photosynthesis to elevated CO_2 concentrations and is this response modulated by soil N supply?; and do functional groupings help explain the variation in species responses to elevated CO_2 and increased soil N?

Materials and Methods

Research site

The study site is located at the Cedar Creek Natural History Area in east central Minnesota, USA (Lat. 45° N, Long. 93° W). The soils are sandy, derived from a glacial outwash sandplain, and previous experiments have determined nitrogen to be the major soil resource that limits plant growth (Tilman, 1987). N mineralization rates for grassland soils at Cedar Creek are estimated to range between 2 and 3 g m^{-2} yr^{-1} (Wedin & Tilman, 1996). Cedar Creek has a continental climate with cold winters (mean January temperature = − 11°C), warm summers (mean July temperature = 22°C), and mean annual precipitation totaling 660 mm yr^{-1}. The average maximum daily temperature and total precipitation for the 1998 and 1999 growing seasons (April–September) was 25°C with 389 mm rainfall in 1998 and 24°C with 637 mm rainfall in 1999.

Experimental design and the FACE system

The overall experiment, referred to as BioCON (Biodiversity, Carbon dioxide, and Nitrogen effects on ecosystem functioning, http://www.swan.lter.umn.edu/biocon/), was established in 1997 on secondary successional grassland after removing previous vegetation (Reich *et al.*, 2001a,b). The study site consisted of six circular areas (20-m diameter), each containing 61–2 × 2 m plots. The experimental treatments were arranged in factorial combination of CO_2 concentration (368 or 560 μmol mol^{-1}) and soil N supply (low or high (4 g N m^{-2} yr^{-1} added)) with each species in monoculture replicated twice for every CO_2 × N level. The design consisted of a split-plot arrangement of treatments in a randomized design with CO_2 treatment as the whole-plot factor, which was replicated three times among the six rings. The subplot factor of soil N treatment was randomly assigned to individual plots among the six rings. CO_2 was applied using free-air CO_2 enrichment (FACE) technology (Lewin *et al.*, 1994) during all daylight hours during the growing season from April 9 to October 16, 1998 and from April 20 to November 9, 1999. One-minute averages were within 10% of the target concentration 94% and 95% of the time in 1998 and 1999, respectively. The high N plots were amended with 4 g N m^{-2} yr^{-1}, as ammonium nitrate (NH_4NO_3) in solid form, in May, June and July of each year. Monoculture plots of 13 species, representing four functional classifications of plants based on similarities in physiology and growth forms: C_3 grasses, C_4 grasses, legumes, and nonleguminous forbs, were chosen for leaf-level physiological measurements in this study. These species include: C_3 grasses: *Agropyron repens* (L.) Beauv., *Bromus inermis* Leyss., *Koeleria cristata* Pers.; C_4 grasses: *Andropogon gerardii* Vit., *Schizachyrium scoparium* (Mich.) Nash, *Sorghastrum nutans* (L.) Nash; nonleguminous forbs: *Achillea millefolium* L.,

Anemone cylindrica A. Gray, *Solidago rigida* L.; and legumes: *Lupinus perennis* L., *Lespedeza capitata* Mich., *Amorpha canescens* Pursh, *Petalostemum villosum* Nutt. Species hereafter are referred to by their genus. Most species were measured in both 1998 and 1999, however, due to low biomass *Lespedeza* was measured only in 1998 and *Amorpha* and *Petalostemum* were measured only in 1999. For plot biomass data refer to Reich *et al.* (2001b).

Gas exchange and leaf nitrogen

During both the 1998 and 1999 growing seasons, *in situ* rates of leaf net photosynthesis (A) were measured using CIRAS-1 portable infrared gas exchange systems (PP Systems, Hitchin, UK) operated in open-configuration with controlled temperature, CO_2 concentration, and vapor pressure. Measurements were made on an upper fully expanded leaf of an individual plant representing each monoculture plot, typically between 09:00 and 15:00 hours local time. Since leaf traits vary with age, all measures were made using leaves of similar ontogenetic stage. We used upper fully expanded young to mid-aged leaves, which corresponded to the period when many leaf traits were relatively stable (Reich *et al.*, 1991). Gas exchange rates of individual leaves of each species were measured on sunny days between June 26 and July 25, 1998 (13 days), and June 12–24, July 9–24, 1999 (14 days). Rates in the 2 years were determined at or near light-saturating conditions (mean PAR ± SE: 1849 ± 18 and 1616 ± 9 μmol m^{-2} s^{-1}, in 1998 and 1999, respectively), at 25.5 ± 0.1 and 26.6 ± 0.1°C (1998 and 1999, respectively), near ambient humidity (mean chamber vapour pressure deficit (VPD) ± SE: 1.65 ± 0.03 and 1.51 ± 0.02 kPa, in 1998 and 1999, respectively), and approximately at the CO_2 concentrations under which the plants were grown (356 ± 1 or 549 ± 1 μmol mol^{-1}). Hereafter, 368 and 560 μmol mol^{-1} will be used to describe both measurement and growth CO_2 concentrations for clarity. Photosynthetic rates were calculated on both leaf area (A, μmol m^{-2} s^{-1}) and leaf mass (A_m, μmol g^{-1} s^{-1}) bases, and will be presented on an area basis unless otherwise denoted. Comparisons made between gas exchange measurements taken at growth CO_2 concentrations are referred to as 'long-term responses' ($A_{@growth}$, A_{560} vs A_{368}). The photosynthetic rate of each sample leaf of plants grown under ambient CO_2 was also determined at 560 μmol mol^{-1} CO_2 concentration to assess the response of species to instantaneous CO_2 enrichment, referred to as 'short-term responses' (A'_{560} vs A_{368}, with A'_{560} referring to ambient grown plants measured at 560). This also allowed the comparison of rates of ambient and elevated CO_2 grown plants at a common CO_2 concentration ($A_{@560}$, A'_{560} vs A_{560}). Each of two replicate plots representing each of the four $CO_2 \times N$ treatment combinations were sampled four times each year resulting in $n = 64$ for each species ($n = 32$ for *Lespedeza*, *Amorpha* and *Petalostemum* measured in one year only). The four samplings of each plot were measured on separate days, at random time points, and were averaged for each replicate plot to incorporate day to day and within day variation in environmental conditions.

The projected areas of leaves used in gas exchange measurements were determined using a digital image analysis program (WinRhizo 3.9, Regent Instruments, Quebec, Canada). Leaves were then oven-dried (65°C) to determine dry mass and specific leaf area (SLA, $cm^{-2}g^{-1}$). Each leaf used in gas exchange measurements was ground and analysed for tissue nitrogen concentration with an NA1500 C-N Analyser (Carlo-Erba Instruments, Milan, Italy). Intrinsic instantaneous water-use efficiency ($A_{@growth}/g_s$) and photosynthetic N-use efficiency (PNUE, $A_{@growth}/N_{area}$) were derived from gas exchange and tissue N data.

Data analysis

Data from four individual leaves, each measured on a separate day, were averaged for each replicate plot to provide an estimate of plot response to the treatment combinations within each year. Therefore, plot was the experimental unit used in ANOVA. For each of the nine variables analysed (Table 2), data from species measured in both 1998 and 1999 were analysed by repeated measures ANOVA including year in the model. Year as a main effect was significant ($P < 0.01$) for seven of the nine variables. The CO_2 treatment × year interaction was not significant ($P > 0.25$) for any variable, however, in three cases the N treatment × year interaction was significant ($P < 0.05$). Because the effect of year was often significant, year × treatment interactions occasionally significant, and most importantly because some species were not measured in both years, we analysed and present statistics and data separately for each year. ANOVA was used in two complementary ways, including either species or functional group as a treatment effect for the following response variables: A, SLA, g_s, $A_{@growth}/g_s$, leaf N concentration (%N) and content (N_{area}, g N m^{-2}), and PNUE. 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 effect of functional group (3 df) was tested against the random effect of species nested within functional group (7 or 8 df). The main effects of species (10 or 11 df) and N (1 df), and all interactions, were tested against the residual error. To test the effect of CO_2 on the relationships between $A_{@560}$-g_s and $A_{m,@560}$-%N we examined all data for individual species using 'separate slopes' analysis of covariance. We tested whether the slopes of regression lines varied among CO_2 treatments. If they did not differ significantly, the interaction term was removed from the model and 'same slopes' analyses were used to test for equality of intercepts between the CO_2 treatment regression lines. All analyses were conducted with statistical analysis software (JMP Version 3.16, SAS Institute Inc., Cary, NC, USA).

Table 1 Interspecific variation in proportional change in area-based net photosynthetic rate in response to elevated compared to ambient CO_2. Mean long-term responses are shown as the ratio of photosynthetic rates determined at growth CO_2 concentrations of 560 vs 368 μmol mol^{-1} (A_{560}/A_{368}). Short-term responses to elevated CO_2 are shown as the ratio of photosynthetic rates of ambient CO_2 grown plants measured at 560 vs 368 μmol mol^{-1} CO_2 (A'_{560}/A_{368}).

	1998				1999			
	Long-term Response (A_{560}/A_{368})		Short-term Response (A'_{560}/A_{368})		Long-term Response (A_{560}/A_{368})		Short-term Response (A'_{560}/A_{368})	
Species	High N	Low N	High N	Low N	High N	Low N	Low N	High N
Forbs								
Achillea	1.0	1.7	1.5	1.6	0.9	1.1	1.5	1.5
Anemone	0.9	1.1	1.5	1.6	0.8	0.8	1.5	1.6
Solidago	0.9	0.9	1.4	1.7	1.1	1.2	1.4	1.4
Legumes								
Lupinus	1.0	0.8	2.1	1.7	1.1	0.9	–[1]	–
Lespedeza	1.1	1.5	1.6	1.5	–	–	–	–
Amorpha	–	–	–	–	1.0	1.0	1.5	1.4
Petalostemum	–	–	–	–	1.1	0.9	1.4	1.3
C_3 Grasses								
Agropyron	1.1	1.1	1.7	1.5	1.4	0.8	1.5	1.4
Bromus	1.3	1.2	1.6	1.6	1.1	1.1	1.4	1.5
Koeleria	1.4	1.3	1.8	1.7	0.8	0.8	1.5	1.5
C_4 Grasses								
Andropogon	0.6	1.2	1.1	1.0	0.9	0.9	1.2	1.2
Schizachyrium	0.9	0.9	1.1	1.1	1.2	1.2	1.1	1.2
Sorghastrum	1.3	0.9	1.2	1.2	1.4	1.0	1.1	1.0

[1]Data not available. A value of 1.0 indicates no difference in rates between CO_2 treatments. Statistics for long-term responses are shown in Table 2. The main effect of measurement CO_2 concentration for the short-term response $P > 0.0001$ in both years and in 1998: N effect $P = 0.99$, species × N $P = 0.14$ 1999: N effect $P = 0.05$, species × N $P = 0.01$, in neither year was measurement CO_2 × N significant.

Results

Net photosynthesis

Mean rates of light-saturated leaf net photosynthesis (A, μmol m^{-2} s^{-1}) of the C_3 species grown at ambient CO_2 (368 μmol mol^{-1}) were increased on average by 64% and 44% in 1998 and 1999, respectively, after short-term exposure to elevated CO_2 (560 μmol mol^{-1}) (A'_{560} vs A_{368}, Table 1). By contrast, photosynthetic rates of C_3 species grown and measured at elevated CO_2 ('long-term exposure') were similar to or only slightly higher (mean difference +13% and +8% in 1998 and 1999, respectively) than plants grown and measured at ambient CO_2 (A_{560} vs A_{368}), and these differences were not statistically significant (Tables 1, 2; Fig. 1). Similarly, short-term exposure to 560 μmol mol^{-1} of C_4 species grown under ambient CO_2 resulted in a 13% increase on average across years, while C_4 plants grown and measured at elevated CO_2 had similar rates of photosynthesis (mean difference −5% and +1% in 1998 and 1999, respectively) as those grown and measured at ambient CO_2 (Tables 1, 2; Fig. 1).

Hence, it appears that marked photosynthetic acclimation to elevated CO_2 occurred in all species in both years. When comparing the very modest proportional change in photosynthetic rates of elevated compared with ambient CO_2 grown plants ('long-term response' A_{560}/A_{368}), with that of the substantial increase in proportional change in response to short-term increases in CO_2 (A'_{560}/A_{368}), pronounced acclimation of photosynthesis to growth under elevated CO_2 was revealed (Table 1). Moreover, comparing elevated and ambient CO_2-grown plants at a common measurement CO_2 concentration (A_{560} and A'_{560}, respectively), acclimation of photosynthesis was indicated by generally lower rates (−27% and −21% in 1998 and 1999, respectively) of elevated grown plants compared with ambient grown counterparts ($A_{@560}$, Table 2, Fig. 2).

In response to soil N treatments in 1998, plants grown under high N had 8% ($P = 0.08$) and 12% ($P = 0.008$) higher photosynthetic rates, on leaf area and mass bases, respectively (Table 2, Fig. 1a). However, not all species responded positively to the high N treatment (Table 2, species × N interaction $P = 0.08$). *Lupinus*, an N-fixing legume, showed an average 34% decrease in $A_{@growth}$ under high N compared with low N (Fig. 1a). During 1999, overall N effects diminished such that $A_{@growth}$ was only slightly to moderately enhanced (+2% on average) in plants grown under high N with species responding similarly (Table 2, Fig. 1b). Although across most species high N increased rates of photosynthesis at least in the first year, the high N treatment had no significant effect on either short- or long-term photosynthetic responses to elevated CO_2. In fact, CO_2 × N interactions were not significant for any response variable analysed (Tables 1, 2).

Table 2 ANOVA probabilities ($P > F$) for treatment (CO_2, N, and species) main effects and interactions on leaf-level traits of grassland species (11 in 1998, 12 in 1999) grown at ambient (368 μmol mol^{-1}) and elevated (560 μmol mol^{-1}) CO_2 concentrations and low N (unamended soil) and high N treatments (addition of 4 g N m^{-2} y^{-1}) over two growing seasons

Main effects & interactions			$A_{@growth}$ (μmol m^{-2} s^{-1})	$A_{m,@growth}$ (nmol g^{-1} s^{-1})	$A_{@560}$ (μmol m^{-2} s^{-1})	SLA (cm^2 g^{-1})	g_s (mmol m^{-2} s^{-1})	$A_{@growth}/g_s$ (mmol CO_2 mol H_2O^{-1})	Leaf % N	Leaf N_{area} (gN m^{-2})	PNUE ($A_{@growth}/N_{area}$) (μmol CO_2 g N^{-1} s^{-1})
CO_2	1998	$P > F$	0.46	0.45	**0.03**	0.98	**0.05**	**0.02**	**0.04**	**0.07**	0.18
		%	(+6)[1]	(+4)	(−28)	(∅)	(−23)	(+37)	(−12)	(−12)	(+13)
	1999	$P > F$	0.25	0.66	**0.01**	0.37	**0.04**	**0.01**	**0.04**	0.21	0.19
		%	(+7)	(+3)	(−21)	(−2)	(−24)	(+43)	(−10)	(−8)	(+8)
N	1998	$P > F$	**0.08**	**0.008**	0.47	0.55	0.64	**0.0003**	**0.0003**	**0.003**	0.57
		%	(+8)	(+12)	(+4)	(+1)	(−3)	(+22)	(+12)	(+13)	(−2)
	1999	$P > F$	0.44	0.13	0.50	0.63	0.86	0.38	0.75	0.66	0.60
		%	(+2)	(+4)	(+2)	(+1)	(+1)	(+4)	(−1)	(−2)	(+3)
Species × CO_2	1998	$P > F$	0.21	0.48	**0.07**	**0.004**	0.93	**0.04**	**0.007**	0.49	0.02
	1999	$P > F$	0.85	0.45	0.26	**0.02**	0.82	0.38	0.25	0.56	0.56
Species × N	1998	$P > F$	**0.08**	**0.0003**	**0.08**	0.58	0.75	**0.003**	0.12	0.75	0.11
	1999	$P > F$	0.24	**0.04**	0.37	**0.07**	0.25	0.78	0.82	0.95	0.18
CO_2 × N	1998	$P > F$	0.30	0.14	0.28	0.88	0.89	**0.09**	0.35	0.56	0.91
	1999	$P > F$	0.10	**0.08**	0.29	0.75	0.81	0.72	0.98	0.54	0.17
CO_2 × N × species	1998	$P > F$	0.19	**0.02**	0.47	0.14	0.28	0.31	0.99	0.99	0.19
	1999	$P > F$	0.57	0.52	0.74	0.63	0.65	0.75	**0.02**	**0.09**	0.12

[1]Percent differences for main effects are shown below P-value. $P < 0.10$ are bold-faced. Species main effect $P < 0.0001$ for all response variables in both seasons. $A_{@growth}$ (area-based photosynthesis at growth CO_2 concentration); $A_{m,@growth}$ (mass-based photosynthesis at growth CO_2 concentration); $A_{@560}$ (area-based photosynthesis measured at common CO_2 concentration of 560 μmol mol^{-1}); SLA (specific leaf area); g_s (stomatal conductance at growth CO_2 concentration); $A_{@growth}/g_s$ (intrinsic instantaneous water-use efficiency); PNUE (photosynthetic N-use efficiency).

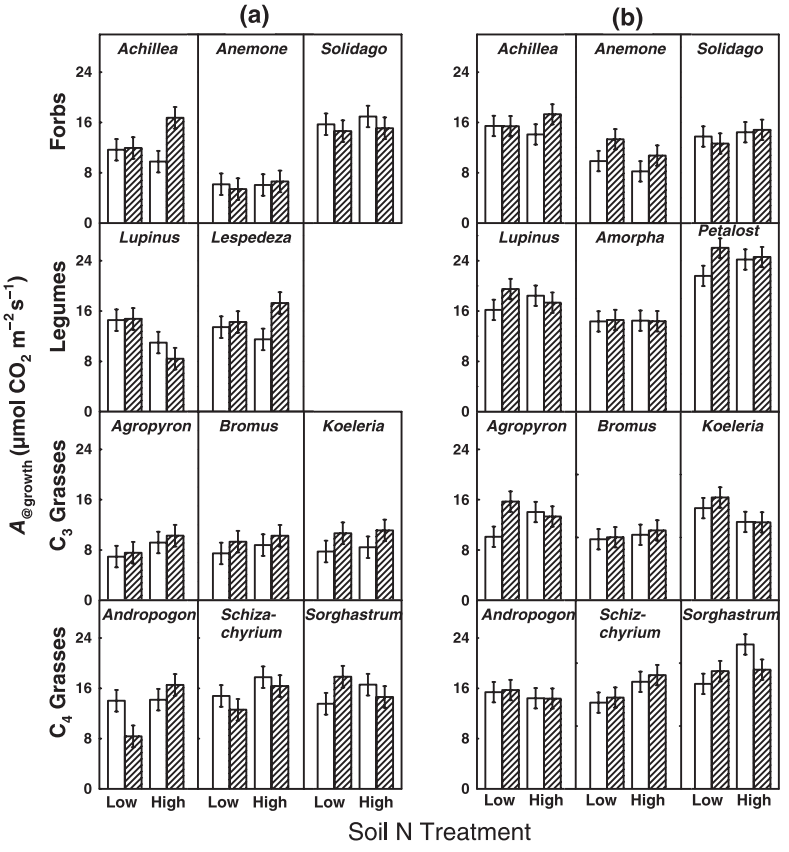

Fig. 1 Area-based rates of leaf net photosynthesis ($A_{@growth}$, μmol CO_2 m^{-2} s^{-1}) of 13 grassland species grown and measured under ambient (open bars, 368 μmol mol^{-1}) and elevated (hatched bars, 560 μmol mol^{-1}) concentrations of CO_2 and low (unamended) and high (amended with 4 g N m^{-2} y^{-1}) soil N. (a) 1998 (11 species) and (b) 1999 (12 species). Shown are least squares means (± SE) from species × CO_2 × N interaction in ANOVA. Species are arranged by functional group. See Table 2 for statistics.

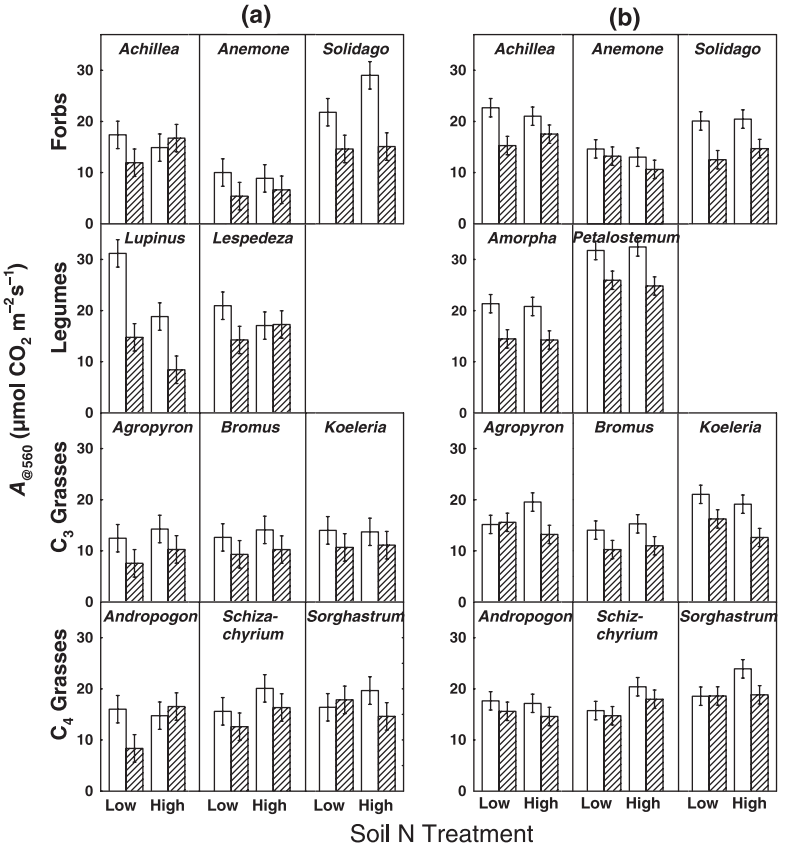

Fig. 2 Area-based rates of net photosynthesis ($A_{@560}$, μmol CO_2 m^{-2} s^{-1}) of 13 grassland species measured at a common CO_2 concentration of 560 μmol mol^{-1}. Plants were grown under ambient (open bars, 368 μmol mol^{-1}) and elevated (hatched bars, 560 μmol mol^{-1}) concentrations of CO_2 and low (unamended) and high (amended with 4 g N m^{-2} y^{-1}) soil N. (a) 1998 (11 species) and (b) 1999 (12 species). Shown are least squares means (± SE) from species × CO_2 × N interaction in ANOVA. Species are arranged by functional group. See Table 2 for statistics.

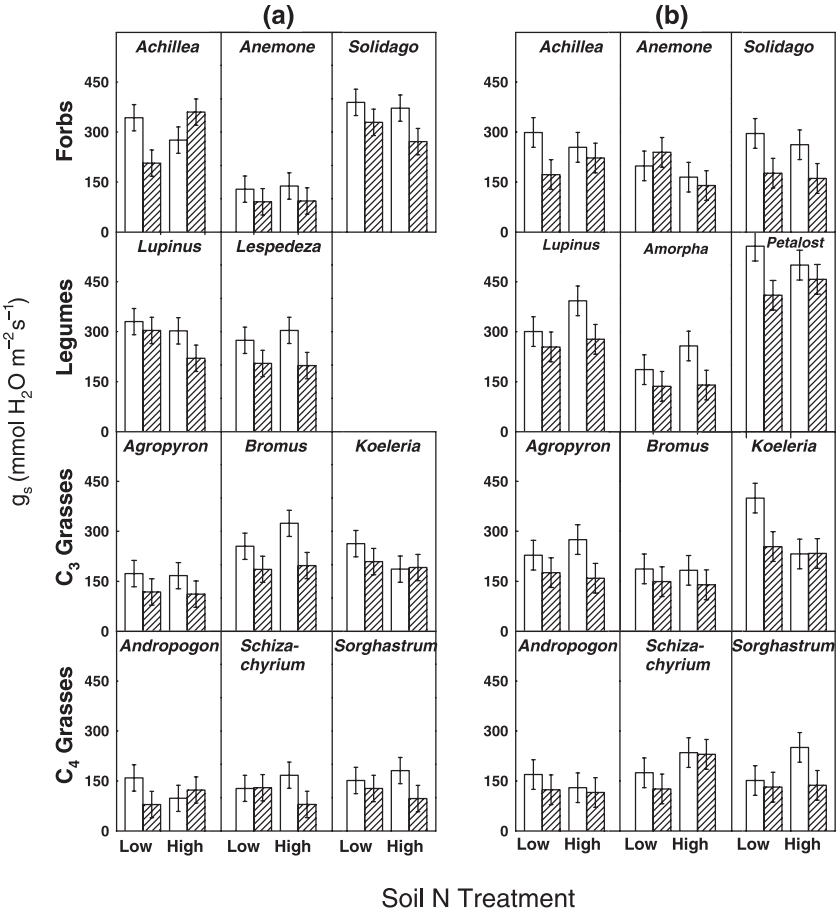

Fig. 3 Leaf stomatal conductance to water vapor (g_s, mmol H_2O m^{-2} s^{-1}) of 13 grassland species grown and measured under ambient (open bars, 368 μmol mol^{-1}) and elevated (hatched bars, 560 μmol mol^{-1}) concentrations of CO_2 and low (unamended) and high (amended with 4 g N m^{-2} y^{-1}) soil N. Shown are least squares means (± SE) from species × CO_2 × N interaction in ANOVA. Species are arranged by functional group. See Table 2 for statistics.

Stomatal conductance and intrinsic instantaneous water-use efficiency

Stomatal conductance to water vapor (g_s) was on average 24% lower across all species and in both 1998 and 1999 in plants grown and measured at elevated compared with ambient CO_2 ($P = 0.05$, Table 2, Fig. 3). The reduced water loss, coupled with slightly enhanced $A_{@growth}$ in elevated CO_2 grown plants, resulted in an average 40% increase ($P = 0.02$) in intrinsic instantaneous water-use efficiency across all species ($A_{@growth}/g_s$, mmol CO_2 mol H_2O^{-1}, Table 2).

High N did not significantly affect stomatal conductance, but in 1998 where high N enhanced net photosynthesis, $A_{@growth}/g_s$ was also increased (+22%, $P = 0.0003$), resulting in one of the few marginally significant interactions between CO_2 and N effects ($P = 0.09$, Table 2). In this case, the response of $A_{@growth}/g_s$ to elevated CO_2 was greater in magnitude under high N (43%) than under low N (23%) (data not shown).

Specific leaf area, leaf nitrogen and photosynthetic nitrogen-use-efficiency

Overall changes in SLA in response to either elevated CO_2 or added N were similar in both years but varied by species (Table 3). Most species showed no difference or slight decreases in SLA between elevated and ambient CO_2-grown plants. Most notable deviations from this pattern occurred in *Andropogon* in 1998 and *Lupinus* in 1999, in which SLA increased under elevated CO_2 by 26% and 17%, respectively, averaged across N treatments. Responses of these two species account for the observed significant species × CO_2 interactions (Table 2).

The effects of CO_2 and N on leaf N concentration (%N) and content (N_{area}, g N m^{-2}) were also independent (Table 2). Across species and years, leaf N declined by roughly 10% in elevated CO_2 grown plants (Table 2, Fig. 4). In 1998, leaf N content increased an average of 13% in leaves of plants grown at high N ($P = 0.003$, Table 2, Fig. 4). However in 1999, leaf N content of plants grown at high N did not differ from their low N grown counterparts ($P = 0.66$, Table 2, Fig. 4).

The effect of elevated CO_2 on leaf N content, coupled with its effect on photosynthesis, resulted in an 11% higher photosynthetic nitrogen-use-efficiency (PNUE, $A_{@growth}/N_{area}$, μmol CO_2 g N^{-1}) on average across species (Table 2). In the first growing season, species PNUE responses to CO_2 enrichment ranged between a 21% decrease to a 59% increase in PNUE (species × CO_2 interaction, $P = 0.02$, Table 2).

Table 3 Specific leaf area (SLA, $cm^2\ g^{-1}$) of grassland species (11 in 1998, 12 in 1999) grown under 368 and 560 μmol mol^{-1} CO_2 and low and high soil N treatments[1]

Functional group	Species	$[CO_2]$	N Treatment			
			1998		1999	
			Low N	High N	Low N	High N
Forbs	*Achillea*	368	111.1	105.0	120.4	112.4
		560	105.7	102.2	106.4	101.5
	Anemone	368	178.8	161.1	149.1	167.2
		560	158.5	165.9	145.4	124.9
	Solidago	368	105.7	109.4	105.2	106.3
		560	104.2	108.1	104.8	113.8
Legumes	*Lupinus*	368	183.3	177.3	193.7	176.7
		560	166.9	189.4	232.1	200.6
	Lespedeza	368	159.7	146.4	– [2]	–
		560	159.9	141.2	–	–
	Amorpha	368	–	–	153.1	169.4
		560	–	–	149.3	145.3
	Petalostemum	368	–	–	114.9	112.5
		560	–	–	95.5	108.0
C_3 Grasses	*Agropyron*	368	195.2	203.0	175.8	187.7
		560	195.9	182.0	164.9	188.4
	Bromus	368	198.6	203.3	166.3	163.0
		560	178.8	223.4	166.7	162.4
	Koeleria	368	138.5	128.5	89.8	102.5
		560	112.8	117.9	86.5	93.5
C_4 Grasses	*Andropogon*	368	225.2	256.7	181.1	189.0
		560	323.2	285.8	185.8	197.3
	Schizachyrium	368	210.3	217.9	204.2	185.7
		560	193.4	221.5	194.6	184.5
	Sorghastrum	368	191.4	207.1	168.1	173.5
		560	195.5	186.7	157.5	174.7

[1]Presented are least squares means (± 12.0 $cm^2\ g^{-1}$ (1998), ± 8.8 $cm^2\ g^{-1}$ (1999)) from species × CO_2 × N interaction in ANOVA. See Table 2 for statistics. [2]Data not available.

$A_{@560}$-g_s and $A_{m,@560}$-%N relationships

We examined the relationships between $A_{@560}$-g_s (Fig. 5a,b) and $A_{m,@560}$-%N (Fig. 5c,d) across species to evaluate possible mechanisms to explain the photosynthetic acclimation to growth under elevated CO_2. As these associations varied among species, four specific cases were chosen that most clearly show the two types of relationships found. Some species grown under elevated CO_2 responded with decreases in photosynthesis that were proportional to decreases in stomatal conductance (Fig. 5a). However in other species, photosynthesis was lower at a given stomatal conductance in elevated compared with ambient CO_2 grown plants (Fig. 5b). Qualitatively, roughly half of the 13 species, in at least one of the 2 yr, showed a relationship between $A_{@560}$ and g_s closer to the former. Declines in photosynthesis of elevated CO_2 grown plants were associated with decreased N concentration in six of the 13 species (Fig. 5c), while the others had lower photosynthetic rates at a given leaf N in elevated compared with ambient CO_2 grown plants (Fig. 5d). Whether species demonstrated one type of relationship vs the other was not related to their functional groupings.

Functional groups

For analyses on data pooled across species within their functional groupings, statistically significant interactions between functional groups and CO_2 or N treatments were few and occurred predominately in 1998 (Fig. 6a,b). In addition, CO_2 and soil N treatment interactions were nonsignificant in all cases. The enhancements in leaf-level photosynthetic rates by growth in elevated CO_2 of the C_3 species (C_3 grasses, legumes, and nonleguminous forbs) were greater (average +10%) than that of the C_4 species (average +1%) across both seasons. The most positive responses were among the C_3 grasses, which had 21% and 11% higher rates of photosynthesis when grown under elevated compared with ambient CO_2 in 1998 and 1999, respectively (Fig. 6a,c).

In terms of leaf N content, there was an interaction between functional groups and CO_2 in 1998 but not 1999. (Fig. 6a,c; $P = 0.02$). The effect of CO_2 concentration on PNUE was also less in the C_4 grasses than the other functional groups in both 1998 and 1999, but this interaction was significant in 1998 only (Fig. 6a, $P = 0.01$). In 1998, the responses of A, $A_{@growth}/g_s$, and leaf N content to the high

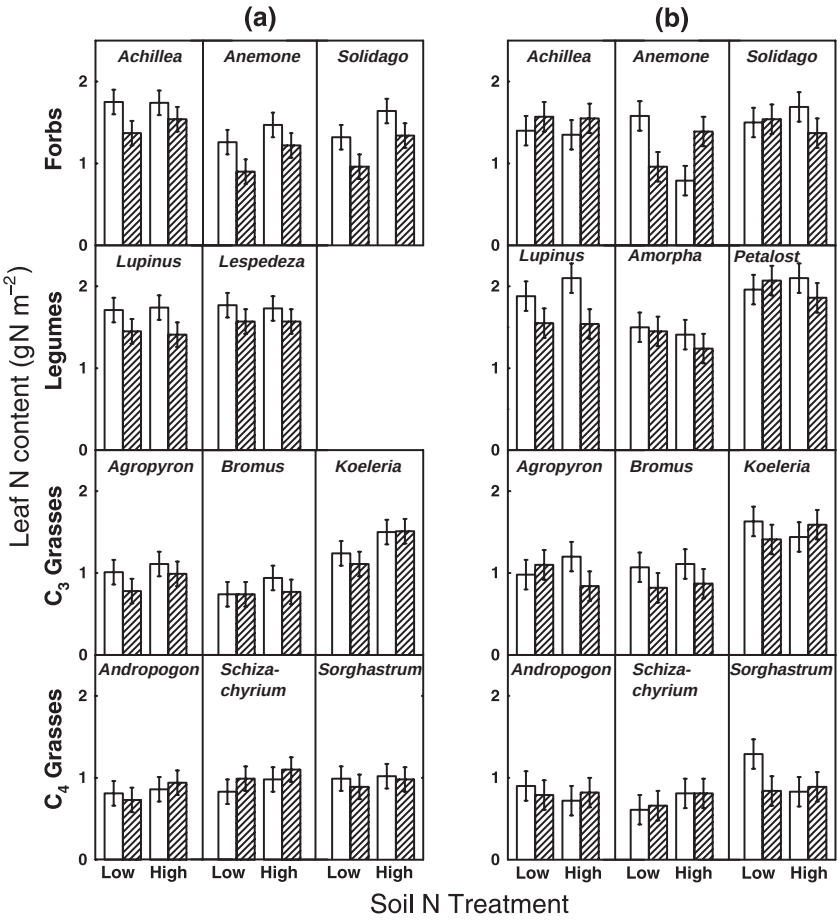

Fig. 4 Area-based leaf N content (N_{area}, g N m^{-2}) of 13 grassland species grown and measured under ambient (open bars, 368 μmol mol^{-1}) and elevated (hatched bars, 560 μmol mol^{-1}) concentrations of CO_2 and low (unamended) and high (amended with 4 g N m^{-2} y^{-1}) soil N. (a) 1998 (11 species) and (b) 1999 (12 species). Shown are least squares means (± SE) from species × CO_2 × N interaction in ANOVA. Species are arranged by functional group. Each year was analysed separately. See Table 2 for statistics.

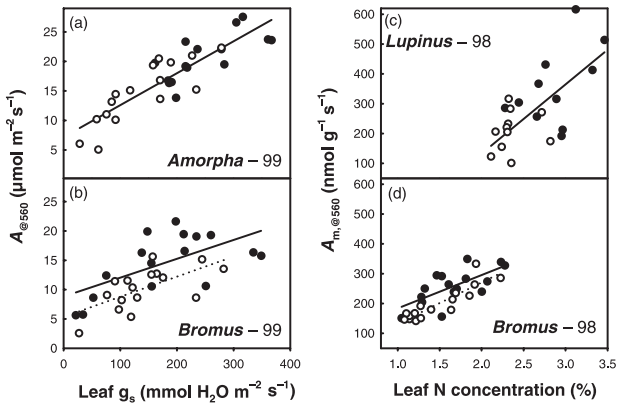

Fig. 5 Relationships between leaf net photosynthesis ($A_{@560}$ or $A_{m,@560}$) and leaf stomatal conductance ($g_{s@560}$, a,b) or leaf N concentration (%, c, d) of foliage grown under ambient (closed circles, 368 μmol mol^{-1}) and elevated (open circles, 560 μmol mol^{-1}) concentrations of CO_2 and measured at a common CO_2 concentration of 560 μmol mol^{-1}. These species were chosen to represent the variation in response across the 13 grassland species included in this study. Shown are data from individual measurements. Coefficients of determination (r^2) for $A_{@560}$ vs g_s: (a) *Amorpha* 99 ($r^2 = 0.75$, $P < 0.0001$) (b) *Bromus* 99 (ambient CO_2 $r^2 = 0.39$, $P = 0.01$; elevated CO_2 $r^2 = 0.41$, $P = 0.007$); for $A_{m,@560}$ vs %N: (c) *Lupinus* 98 ($r^2 = 0.46$, $P = 0.0004$) (d) *Bromus* 98 (ambient CO_2 $r^2 = 0.47$, $P = 0.003$, elevated CO_2 $r^2 = 0.79$, $P < 0.0001$). See text for further explanation of analysis.

soil N treatment were positive in each functional group but the legumes responded in the opposite direction (fxgrp × N interactions, Fig. 6b). The few cases in which species pooled by functional groups showed differential responses to CO_2 concentration or soil N treatments were due to either the legumes or the C_4 grasses.

Discussion

Acclimation of photosynthesis to elevated CO_2 concentration and increased soil N

Photosynthetic rates are well known to increase in C_3 plants in response to short-term increases in CO_2 concentrations (Bazzaz, 1990; Drake *et al.*, 1997). In this study, photosynthetic rates increased an average of 55% for the C_3 species and 13% for the C_4 species in response to short-term CO_2 enrichment, which is comparable in magnitude with the 60% average increase found in other studies of C_3 species (Strain & Cure, 1994; Curtis, 1996; Drake *et al.*, 1997). Theory based on simple CO_2 diffusion into C_3 leaves suggests that a photosynthetic enhancement of 55% is expected with +200 μmol mol^{-1} enrichment in CO_2 (as in this study) and no photosynthetic acclimation (Katul *et al.*, 2000).

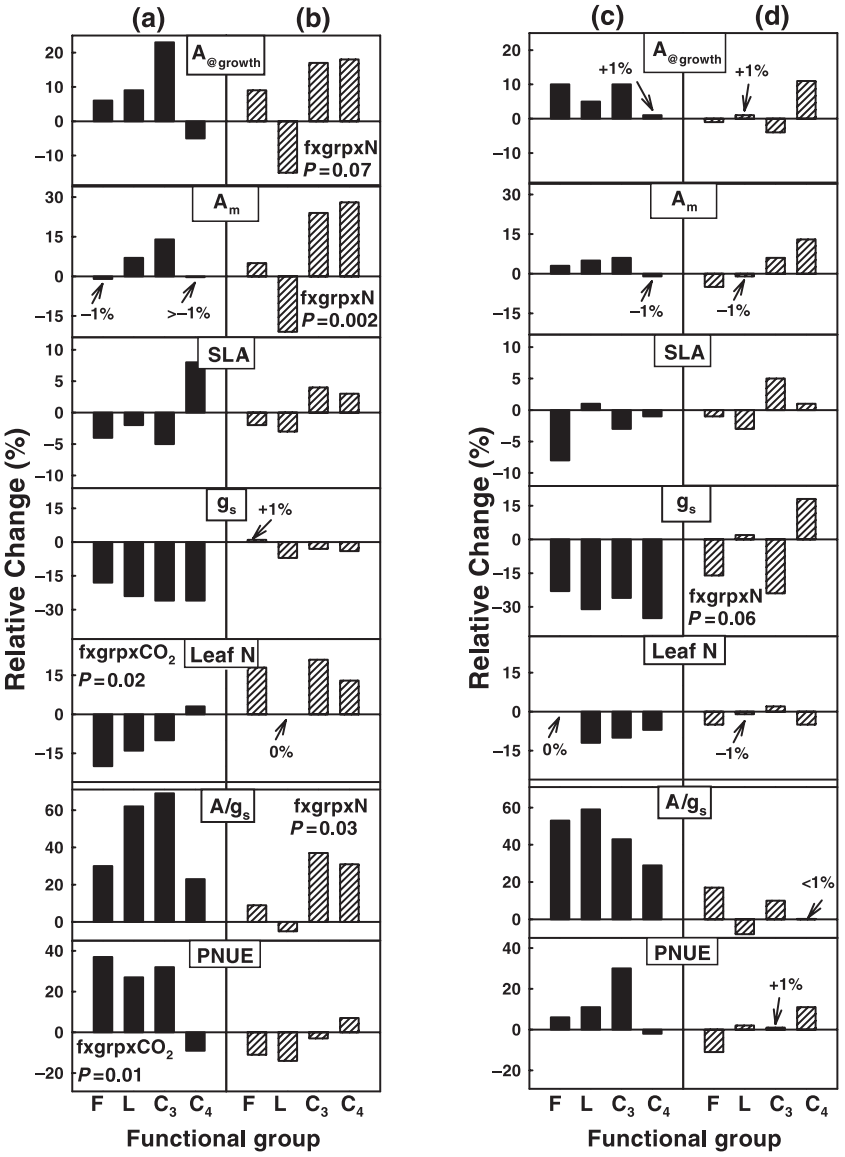

Fig. 6 Relative percent changes in response traits by functional groupings ((elevated – ambient)/ambient × 100). F, forbs; L, legumes; C_3, C_3 grasses; C_4, C_4 grasses; (a) and (b) 1998 (c) and (d) 1999; (a) and (c) CO_2 treatment effects pooled across N treatments (black bars) (b) and (d) N treatment effects pooled across CO_2 treatment (hatched bars). Significant functional group × CO_2 treatment (fxgrp × CO_2) and functional group × N treatment (fxgrp × N) interactions are given. No CO_2 × N interactions were significant. $A_{@growth}$ (area-based photosynthesis, μmol m^{-2} s^{-1}), A_m (mass-based photosynthesis, nmol g^{-1} s^{-1}), specific leaf area (SLA, cm^2 g^{-1}), g_s (stomatal conductance, mmol m^{-2} s^{-1}), Leaf N (gN m^{-2}), A/g_s (intrinsic instantaneous water-use efficiency, mmol CO_2 mol H_2O^{-1}), PNUE (photosynthetic N-use efficiency, μmol CO_2 g N^{-1} s^{-1}).

The degree of relative photosynthetic responsiveness to elevated CO_2 over longer-terms varies substantially. Recent reviews summarize long-term photosynthetic enhancements in response to growth under elevated CO_2 on the average of 30% and 24% in C_3 and C_4 Poaceae species, respectively (Wand *et al.*, 1999), and 50% and 51% across 41 woody C_3 species (Curtis, 1996), and 15 field-based studies on forest tree species (Medlyn *et al.*, 1999), respectively. The long-term enhancement of photosynthesis by elevated CO_2 we observed was modest and not statistically significant in either year. C_3 species on average increased photosynthesis when grown under elevated CO_2 by 13% and 8%, in 1998 and 1999, respectively, and C_4 species demonstrated negligible responses in both years. This is in contrast to many field-based studies that have found strong and persistent stimulation of photosynthetic rates in C_3 species grown under elevated CO_2 over one to three growing seasons (Ellsworth *et al.*, 1995; Jackson *et al.*, 1995; Drake *et al.*, 1996; Stirling *et al.*, 1997; Bryant *et al.*, 1998; Curtis *et al.* 2000) as well as positive enhancements for C_4 species (Read *et al.*, 1997; Wand *et al.*, 1999). However, other studies demonstrate limited stimulation of photosynthesis of plants grown for weeks to months under elevated CO_2 (Tissue & Oechel, 1987; Li *et al.*, 1999; Roumet *et al.*, 2000) or a loss of the initial stimulation in photosynthesis over time (Oechel *et al.*, 1994; Körner *et al.*, 1997).

Species variation in responsiveness of photosynthesis to elevated CO_2 can be explained by differences in the extent of photosynthetic acclimation in plants grown under elevated CO_2. In our study on nutrient poor soil, species on average demonstrated 80% acclimation of photosynthesis to elevated atmospheric CO_2 concentrations as judged by comparing the magnitude of long-term photosynthetic enhancements with

elevated CO_2 (A_{560} vs A_{368}) to the photosynthetic response to short-term exposure to elevated CO_2 (A'_{560} vs A_{368}). This is much larger than shown in most previous studies (Curtis, 1996; Medlyn *et al.*, 1999; Wand *et al.*, 1999).

Is photosynthetic acclimation to elevated CO_2 modulated by soil N supply? The magnitude of the CO_2 effect on gas exchange and other leaf traits in these 13 grassland species was apparently independent of soil N supply in these two years. In only three specific cases (*Achillea* 98 and 99, and *Lespedeza* 98, Fig. 1) was there an increase in magnitude of enhancement of photosynthesis due to elevated CO_2 under high N, though not statistically significant (Table 2). Several other studies have found plants to be comparably responsive to CO_2 at both high and low nutrient concentrations (Hättenschwiler & Körner, 1996; Lloyd & Farquhar, 1996; Körner *et al.*, 1997; Cotrufo *et al.*, 1998). However, many studies have found greater photosynthetic responsiveness to elevated CO_2 at higher N availability (Curtis, 1996; Miglietta *et al.*, 1996; Rogers *et al.*, 1998; Sims *et al.*, 1998; Wolfe *et al.*, 1998; Weerakoon *et al.*, 1999; Curtis *et al.*, 2000). Moreover, in some cases, there is evidence of acclimation of photosynthesis under CO_2 enrichment only under certain conditions such as low nutrient supply (Jones *et al.*, 1996; Miglietta *et al.*, 1996; Rogers *et al.*, 1998, Sims *et al.*, 1998, Weerakoon *et al.*, 1999). In our study, the positive effects of high N on traits such as net photosynthetic rates, $A_{@growth}/g_s$, and leaf N concentrations were found only in the first growing season and diminished by the second year. While this difference in response between two years does not necessarily constitute a trend, it was contrary to expectations that the effects of adding N into the system each year would result in stronger N effects over time. This minimal N effect may have contributed to the lack of a $CO_2 \times N$ interaction.

One of the most consistent responses across species and growing seasons in our study was a decline in stomatal conductance to water vapor (g_s). All species decreased g_s by an average 24% when grown under elevated CO_2. While some studies report little or inconsistent effects of elevated CO_2 on g_s (Gunderson & Wullschleger, 1994; Ellsworth *et al.*, 1995; Curtis, 1996; Stirling *et al.*, 1997) the more common response is a decline in g_s comparable in magnitude to this study (Roumet *et al.*, 2000). Reviews cite average declines in g_s of 24% and 29% in C_3 and C_4 Poaceae species, respectively (Wand *et al.*, 1999), 23% across 23 tree species (Field *et al.*, 1995), and 34% across crop species (Kimball & Idso, 1983) in elevated compared with ambient CO_2 grown plants.

As leaf N concentration declined an average of 13%, carbon assimilation expressed per unit leaf N (i.e. PNUE) increased an average of 11% across the species in this study. However, this response was not statistically significant and not consistent across all species. Cotrufo *et al.* (1998) found a comparable reduction in tissue N concentrations (average of 14%) of elevated CO_2 grown C_3 and C_4 plants across 75 published studies and other studies have also found that PNUE increases in response to growth under elevated CO_2 (Bryant *et al.*, 1998; Tjoelker *et al.*, 1998; Peterson *et al.*, 1999b; Curtis *et al.*, 2000), however, in some cases this did not occur consistently (Roumet *et al.*, 2000).

Several possible explanations for the acclimation of photosynthesis in plants grown under elevated CO_2 over longer terms have been proposed. Hypotheses include possible stomatal limitations of photosynthesis due to reduced g_s (Drake *et al.*, 1997), or nonstomatal limitations such as reduced tissue N concentrations potentially leading to a reduced photosynthetic capacity in plants grown under elevated CO_2 (Peterson *et al.*, 1999), or a potential feedback inhibition of photosynthesis induced by an accumulation of excess carbohydrates (Farrar & Williams, 1991; Stitt, 1991).

We examined the relationships between $A_{@560}$-g_s and $A_{m,@560}$-%N to test the first two hypotheses. Results varied among species, but did provide evidence for both possible mechanisms. Some species grown under elevated CO_2 responded with decreases in photosynthesis that were proportional to decreases in g_s (Fig. 5a). In these cases, there was a concomitant decrease in $C_i : C_a$ (data not shown) further suggesting that the decline in g_s of elevated CO_2 grown plants was associated with a lower intercellular CO_2 supply and reduced photosynthetic rates. In other species, photosynthesis was lower at a given g_s, with similar or slightly higher $C_i : C_a$, in elevated compared with ambient CO_2 grown plants (Fig. 5b), suggesting that nonstomatal limitations are also involved.

A potential nonstomatal limitation involves the commonly strong relationship between tissue N and photosynthesis. Regarding the N hypothesis to explain photosynthetic acclimation, we did find a decline in photosynthesis in elevated compared with ambient CO_2 grown plants in proportion to the change in leaf N concentration (Fig. 5c) in roughly half the species. Thus CO_2-induced decreases in leaf N concentration are associated with reduced photosynthetic potential probably via changes in N-rich photosynthetic enzymes that are reflected in total leaf N. However, in other cases, photosynthesis was lower at a given leaf N in elevated compared with ambient CO_2 grown plants (Fig. 5d) suggesting that stomatal or other nonstomatal limitations explain the observed acclimation in such cases.

Studies have found that an increase in total nonstructural carbohydrates (TNC) correlates with decreased photosynthesis in elevated CO_2 grown plants (Tjoelker *et al.*, 1998; Roumet *et al.*, 2000), however, others report increased TNC without a decrease in photosynthetic enhancement (Wullschleger *et al.*, 1992; Will & Ceulemans, 1997). Total nonstructural carbohydrates determined from leaves collected from the same plots used for gas exchange in this study in 1999, indicate an overall 24% greater TNC concentration in elevated CO_2 grown foliage, but this varies substantially across species (M. G. Tjoelker *et al.*, unpublished). Because acclimation of photosynthesis occurs across all the species in the study, but not all species increased TNC when grown under elevated

compared with ambient CO_2, these data are not sufficient to support or refute this as a possible explanation of the photosynthetic acclimation seen in this study.

Most species showed intermediate responses with respect to the examples shown in Fig. 5. Considering this evidence of stomatal and nonstomatal limitations on photosynthesis, no single mechanism explains the magnitude of photosynthetic acclimation seen across all the species in this study, and the data suggests that even within a single species a combination of these mechanisms are possible. Thus, we can ascribe the low or negligible photosynthetic enhancement observed at our site to the combined effects of decreased g_s, decreased leaf N concentration, and possibly increased TNC in response to growth under elevated CO_2.

Functional groups

In our study, the grouping of species into the functional classifications: C_3 grasses, C_4 grasses, legumes, and nonleguminous forbs, are based on discrete physiological and growth form traits commonly used to group species. Our objective was to evaluate whether these categories are helpful in explaining variation in species response to elevated CO_2 and increased N supply across the 13 perennial prairie species in this study. The C_4 grasses are grouped owing to their photosynthetic pathway that concentrates CO_2 in bundle sheath cells, which effectively increases the concentration of CO_2 at the site of carboxylation and therefore, presumably results in near saturation at current CO_2 levels. As predicted, the C_4 species were less responsive than the C_3 species to elevated CO_2 over the long-term with negligible effects on photosynthesis compared with a 10% average increase for all the C_3 species combined. The legumes, unique in the ability to symbiotically fix N_2, responded to the high N treatment oppositely in terms of net photosynthesis, A/g_s, and tissue N relative to the other functional groups. However, species pooled by functional groups responded differently to elevated CO_2 or high soil N treatments in relatively few cases, only for the C_4 grasses and the legumes, and predominantly in the first year of growth under CO_2 and N treatments. In a companion study by Reich *et al.* (2001b), which looked at plot level traits such as total biomass, total plant N, soil solution N and soil water on these same monoculture plots, it was also found that in general, C_4 grasses were less responsive to elevated CO_2 than all C_3 species as a group and legumes were less responsive to the N treatment. Overall, the variation in species responsiveness to elevated CO_2 and soil N supply was generally unrelated to their functional groupings.

Summary

For this diverse set of species with varying ecophysiology, elevated CO_2 only modestly stimulated photosynthesis over the longer term, and not in all cases. However, rates of stomatal conductance declined consistently across species. In general, soil N supply did not modulate species responses to elevated CO_2. In addition, the variation in species response was not exclusively explained by their functional groupings suggesting that current functional classifications may not be sufficient for understanding leaf-level physiological responses to elevated CO_2 and N. The extent to which wild species acclimate photosynthesis to elevated CO_2, and how the efficiency of resource use is affected by growth under elevated CO_2, appear to be critical in determining plant growth responses to changing atmospheric CO_2 and N deposition.

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