Legume species identity and soil nitrogen supply determine symbiotic nitrogen-fixation responses to elevated atmospheric [CO$_2$]

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Summary

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• In nitrogen (N)-limited systems, the response of symbiotic N fixation to elevated atmospheric [CO$_2$] may be an important determinant of ecosystem responses to this global change. Experimental tests of the effects of elevated [CO$_2$] have not been consistent. Although rarely tested, differences among legume species and N supply may be important.
• In a field free-air CO$_2$ enrichment (FACE) experiment, we determined, for four legume species, whether the effects of elevated atmospheric [CO$_2$] on symbiotic N fixation depended on soil N availability or species identity. Natural abundance and pool-dilution ^{15}N methods were used to estimate N fixation.
• Although N addition did, in general, decrease N fixation, contrary to theoretical predictions, elevated [CO$_2$] did not universally increase N fixation. Rather, the effect of elevated [CO$_2$] on N fixation was positive, neutral or negative, depending on the species and N addition.
• Our results suggest that legume species identity and N supply are critical factors in determining symbiotic N-fixation responses to increased atmospheric [CO$_2$].

Key words: carbon dioxide, legumes, nitrogen fertilization, nitrogen fixation, symbiosis.

Introduction

Because of their symbiotic relationship with bacteria that reduce atmospheric N$_2$ to NH$_3$, legumes may be less N limited, and may therefore exhibit a greater productivity response to elevated [CO$_2$], than species that do not fix N. Indeed, several studies report growth stimulation of legumes by elevated [CO$_2$] (Soussana & Hartwig, 1996; Zanetti *et al.*, 1997; Teyssonneyre *et al.*, 2002; Lee *et al.*, 2003a), as well as [CO$_2$]-induced stimulation of N fixation (Hungate *et al.*, 1999; Feng *et al.*, 2004). In addition to its direct effect on legume productivity, this response might have implications for other species, as legumes could, in turn, enhance the responses of co-occurring species to elevated [CO$_2$] by increasing soil N availability (Zanetti *et al.*, 1997; Hartwig *et al.*, 2002;

but see Lee *et al.*, 2003b). If legumes alleviate N limitation, communities with legumes might support a greater number of N-demanding species under elevated atmospheric [CO$_2$] than those that lack legumes (Körner, 2000).

Although positive productivity responses to [CO$_2$] increases are common, results published to date are not universally consistent with the theoretical prediction that legumes will increase their rates of N fixation as a result of elevated [CO$_2$] (e.g. Arnone, 1999; Leadley *et al.*, 1999; Niklaus *et al.*, 2001; Vitousek *et al.*, 2002; Hungate *et al.*, 2004). It remains unclear why some studies have shown stimulation of N fixation by elevated [CO$_2$] and others have not. One possible source of variation is the availability of nitrogen itself. As legume species rarely rely exclusively on atmospherically fixed N, legume N-fixation rates may be dependent on soil N supply, although

N fixation may not necessarily be reduced by N addition (Pearson & Vitousek, 2001).

A second source of variation in legume response to atmospheric [CO_2] might be differences among legume species. Although the importance of species differences to ecosystem processes is widely recognized (Wedin & Tilman, 1990; Hobbie, 1992; Hooper & Vitousek, 1997; Hooper & Dukes, 2004), little attention has been paid to potential differences among legume species in their response to environmental change (but see Leadley *et al.*, 1999). If species vary in their relative reliance on soil N, its availability might modulate the legume response to [CO_2] (Høgh-Jensen & Schjoerring, 1997; Lee *et al.*, 2003a). In addition, legume–rhizobium relationships can be highly species-specific, and fixation rates can be strongly dependent on both legume species and bacterial strain (Shabayev *et al.*, 1996; Provorov & Tikhonovich, 2003). If legumes differ in their N requirements and relationships with N-fixing bacteria, these species could exhibit a range of dependence on bacterial N fixation. In N-limited, legume-rich plant communities, such as grasslands and savannas, these differences might also contribute to compositional shifts over time as the ecosystem N availability changes. To our knowledge, in spite of its potential importance to ecosystem responses, there have been few attempts to compare the N-fixation responses of multiple legume species to elevated [CO_2] or N. In this study we employed $\delta^{15}N$ natural abundance and ^{15}N pool-dilution methods to determine whether the response of symbiotic N fixation to N availability and atmospheric [CO_2] varied among four prairie legume species. If the N-fixation response to elevated [CO_2] differs across species, and with N availability, we would expect to see evidence of that in plant performance. Therefore, we also determined whether the above-ground productivity of these four species responded in a similar manner as N fixation to these global change factors.

Materials and Methods

Experimental design

This study was conducted within a larger experiment (BioCON; http://www.lter.umn.edu/biocon; see Reich *et al.*, 2001a) located at the Cedar Creek Natural History Area in east central Minnesota, MN, USA (lat 45° N; long 93° W). BioCON has been designed to address the interacting effects of biodiversity, elevated atmospheric [CO_2], and N fertilization on grassland ecosystem function. Briefly, it consists of six 20-m diameter 'rings' – three at ambient [CO_2] (368 µmol mol^{-1}) and three at elevated [CO_2] (560 µmol mol^{-1}) – using a free-air CO_2 enrichment (FACE) system (Lewin *et al.*, 1994). Each ring contains 61 individual 2×2 m plots. Half of those plots, selected at random, receive the equivalent of 4 g of N (NH_4NO_3) m^{-2} yr^{-1}. We employed the subset of plots planted in monoculture of this larger experiment for our study (two plots of each species per combination of [CO_2]

and N levels). Seeds of each species were planted in 1997, and treatments were initiated in 1998. The four legume species studied were *Amorpha canescens* Pursh, *Lespedeza capitata* Mich., *Lupinus perennis* L., and *Petalostemum villosum* Nutt. [= *Dalea villosa* (Nutt.) Spreng.]. Species hereafter are referred to by genus only. The other species in the experiment include four species each of C_3 non-leguminous forbs, C_3 grasses, and C_4 grasses. These non-legumes were used to provide a non-fixing reference value for the $\delta^{15}N$ estimation of N fixation (as described in $\delta^{15}N$ analyses).

The experimental design was a split-plot, with [CO_2] treatment as the main-plot factor and ring as the subplot. Except where stated otherwise, data were analysed by using a split-plot, mixed model analysis of variance (ANOVA) (ring as random effect; [CO_2], N and species as fixed effects), and were transformed as necessary to meet the assumptions of ANOVA. All analyses were performed by using SAS for Windows (version 8.02; The SAS Institute, Cary, NC, USA).

$\delta^{15}N$ analyses

In June 2002, green leaves of all legume species and all reference plants were collected, dried, ground to a fine powder and analysed for total N and $\delta^{15}N$ (ThermoFinnigan Delta Plus mass spectrometer; Kansas State University Stable Isotope Mass Spectrometry Laboratory, Manhattan, KS, USA). $\delta^{15}N$ is expressed as 'per mil' relative to atmospheric N_2 [$(R_{sample}/R_{standard} - 1) \times 1000$, where R is the ratio of $^{15}N : ^{14}N$, and the concentration of atmospheric ^{15}N is 0.366%]. These data permitted an estimation of the percentage of leaf N that originated from soil pools vs the amount that is obtained from atmospheric N via rhizobial N fixation (Shearer & Kohl, 1991). Our approach followed that described by Shearer & Kohl (1991, see also Handley & Scrimgeour, 1997), where the proportion of N derived from the atmosphere (Ndfa) is:

$$\text{Ndfa} = \frac{\delta^{15}N_{\text{soil-derived N}} - \delta^{15}N_{\text{N-fixing plant}}}{\delta^{15}N_{\text{soil-derived N}} - \delta^{15}N_{\text{fixed N}}}$$

Non-legume species with similar growth forms growing in the same soil as the legume of interest were used to estimate $\delta^{15}N_{\text{soil-derived N}}$. Thus, a central assumption of this method is that the $\delta^{15}N$ of the non-legume species is an accurate representation of soil-derived N in the legumes. Because we lack detailed knowledge of soil N preferences of these species, our estimate of soil-derived N was therefore obtained from a mean $\delta^{15}N$ of all of the other 12 non-leguminous species in monoculture in each treatment combination ([CO_2] and N). Although the species in this experiment are relatively similar morphologically (all herbaceous perennials), this potentially introduces error by including species that are significantly different from the legumes in terms of rooting patterns and N preferences. Therefore, we also present the variation in the non-legume $\delta^{15}N$ values and evaluate the effect of that

variation on our estimates of Ndfa by calculating Ndfa based on the 12-species mean $\pm$ 1 standard error (SE) (see the results).

The $\delta^{15}N$ of N derived from fixation ($\delta^{15}N_{\text{fixed N}}$ or 'B-value') can be depleted more than atmospheric $\delta^{15}N$ (= 0) and is often between -1 and -2‰ (Shearer & Kohl, 1991). This depletion is attributed to discrimination against the heavier isotope during N fixation and transfer to the plant. Therefore, an additional uncertainty associated with all studies that employ this method is the extent to which ^{15}N is being discriminated against during the fixation of N by bacteria and its subsequent transfer to the plant. Because we did not quantify B-values, we set this parameter for each species to the most negative $\delta^{15}N$ obtained for all N-fixing plant species (Eriksen & Høgh-Jensen, 1998; Riffkin et al., 1999; Hansen & Vinther, 2001). As in all studies that use this approach, this estimate of the B-value makes the explicit assumption that at least one individual is receiving 100% of its N from fixation, making these estimate of Ndfa potentially inflated if no individuals sampled rely on only atmospheric sources. We then used a bootstrapping approach to determine how uncertainty in the value of B would affect the significance of [CO_2], N, species identity and their interactions in our models. We first constructed a normal distribution of B-values from published sources for agricultural and nonagricultural species (see Fig. 1 and the Appendix for references). These B-values were obtained from greenhouse experiments typically with known inocula and represent a distribution of B-values that are potentially valid for the species used in our experiment. This distribution was constrained for each species, such that the Ndfa was never greater than one (i.e. 100% N from fixation), by truncating the upper end of the B-value distribution at the minimum (most negative) $\delta^{15}N$ value observed for that species.

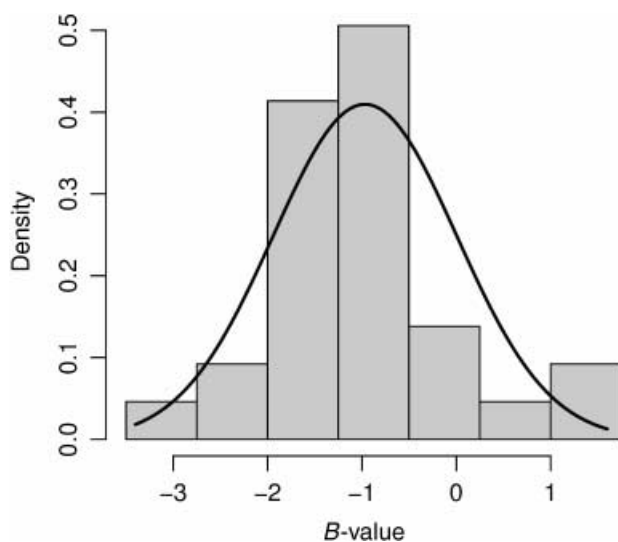We then used resampling to determine how uncertainty in B affected the parameter estimates. For each bootstrap run we randomly sampled four B-values (one for each species) from the normal distribution constructed from literature values, and calculated Ndfa for all plots based on these species-specific B-values. Next, we used a mixed-model ANOVA to test for the effects of [CO_2], N, and species on N fixation. This procedure was repeated 1000 times. We report the proportion of bootstraps that resulted in significant ($P < 0.05$) treatment effects.
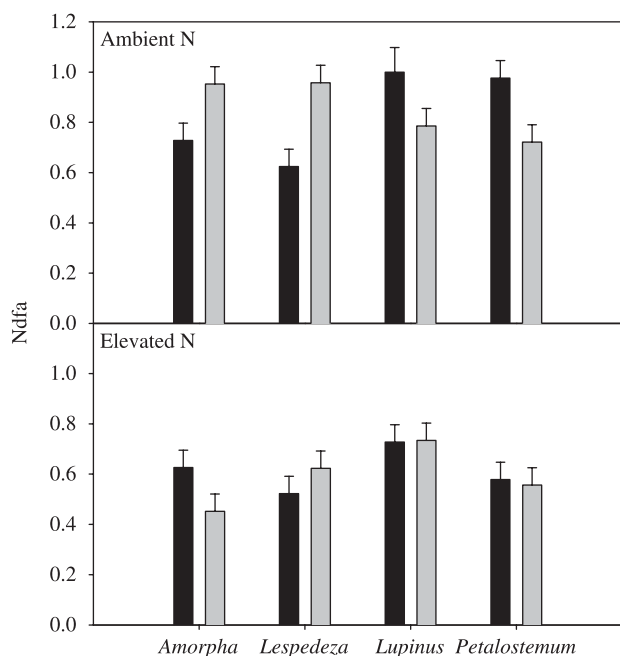
The fertilizer N had an enriched ^{15}N signature (0.3850 atom% ^{15}N) that was evident in all plants treated with elevated N. The use of the average reference plant to estimate soil $\delta^{15}N$ across N treatments incorporates this signal and allows direct comparisons to be made of N fixation between N treatments by using the 'natural abundance' method. Because of this enrichment, we were also able to calculate the proportion of N derived from fixation and from the fertilizer for the elevated N treatment by using a ^{15}N pool-dilution method in the elevated N treatment (Danso et al., 1993; Blumenthal & Russelle, 1996; Lee et al., 2003a). The estimate of Ndfa by this pool-dilution method agreed well with the value obtained by using the natural abundance method ($R^2 = 1$, slope = 1.05, data not presented).

Plant productivity

Within each plot, above- and below-ground biomass was harvested in June and August of 2002. Above-ground biomass was estimated by clipping 0.1-m^2 plots and weighing the dried biomass. Litter (the previous year's growth) was sorted from the current year's production and weighed separately. We report here the above-ground productivity results from 2002 to determine whether there was any relationship between our estimates of N fixation and productivity. As these species vary substantially in their phenology, and dead biomass can be lost between harvests, we analysed and report the peak above-ground biomass by plot.

Results

The legume species exhibited a range of $\delta^{15}N$ values. The lowest values observed by species (i.e. the species-specific B-values) were: Amorpha, -1.2‰; Lespedeza, -2.1‰; Lupinus, -1.7‰; and Petalostemum, -1.0‰. Estimates of soil-derived $\delta^{15}N$ values for each treatment (mean $\pm$ 1 SE), based on the 12 non-leguminous species, were: 0.0‰ $\pm$ 0.1 (ambient CO_2 – ambient N), 0.6‰ $\pm$ 0.3 (elevated CO_2 – ambient N), 41.1‰ $\pm$ 2.4 (ambient CO_2 – elevated N), and 39.3‰ $\pm$ 3.7 (elevated CO_2 – elevated N). In order to evaluate the effect of variation in these estimates of soil-derived N, we calculated the Ndfa value based on the mean soil-derived $\delta^{15}N$ value minus 1 SE and then on the mean plus 1 SE for each treatment combination. This resulted in an overall divergence

Fig. 1 Distribution of B-values obtained from published studies ($n = 58$; see the Appendix for sources).

Fig. 2 Proportion of leaf N derived from atmospheric N$_2$ fixation in legumes grown at ambient and elevated atmospheric [CO$_2$] and N (data shown are the least-squares means + 1 standard error). Ambient [CO$_2$], black bars; elevated [CO$_2$], grey bars. Values are based on species-specific δ^{15}N values for N derived from the atmosphere, a δ^{15}N value derived from 12 other non-leguminous species in the each of the four treatment combinations for N derived from soil, and legume leaf δ^{15}N. See Table 1 for statistical analysis. Ndfa, proportion of N derived from the atmosphere.

Table 1 Results of a mixed-model analysis of variance (ANOVA) of the effects of species, [CO$_2$] and nitrogen treatments on the proportion of leaf N derived from the atmosphere

Source	F-value (degrees of freedom)	P
[CO$_2$]	0.00 (1,4)	0.993
N	42.79 (1,11)	< 0.001
[CO$_2$] × N	0.41 (1,11)	0.537
Species	2.52 (3,11)	0.112
[CO$_2$] × species	4.87 (3,11)	0.022
N × Species	0.76 (3,11)	0.537
[CO$_2$] × N × species	4.60 (3,11)	0.025

Analysis was based on δ^{15}N of four prairie legume species and 12 non-leguminous species grown at ambient (368 µmol m^{-2} s^{-1}) and elevated (560 µmol m^{-2} s^{-1}) [CO$_2$] and ambient and elevated (+4 g of N m^{-2} yr^{-1}) N.

Table 2 Results of bootstrap analysis of the sensitivity of the proportion of N derived from the atmosphere (Ndfa) to variation in the δ^{15}N of atmospherically fixed N (equivlant to the B-value; see the Materials and Methods for a detailed explanation of the analysis)

Bootstrap	[CO$_2$] × species	N × species	[CO$_2$] × N × species
All samples	0.85	0.55	0.70
When three-way interaction is not significant	0.64	0.76	

Values represent the proportion of runs with significant interactions in a mixed-model analysis of variance (ANOVA) from 1000 bootstraps. Across all runs, ≈ 95% resulted in significant interactions with species.

from our mean Ndfa estimate of approx. ± 0.02. Leaf N concentration also varied significantly among species ($F_{3,11}$ = 6.89, P = 0.008), but did not respond to the treatment with [CO$_2$] or N (N% ± 1 SE: *Amorpha*, 2.1 ± 0.1; *Lespedeza*, 1.8 ± 0.1; *Lupinus*, 1.4 ± 0.1; *Petalostemum*, 1.9 ± 0.1).

The Ndfa exhibited a complex, three-way interactive response to species identity, atmospheric [CO$_2$] and N fertilization in this experiment (Fig. 2, Table 1). There was a significant decrease in Ndfa with N fertilization across species. However, the effect of [CO$_2$] depended strongly on both N fertilization and species identity. Within the ambient N treatment, *Amorpha* and *Lespedeza* showed increases in Ndfa with elevated [CO$_2$], whereas *Lupinus* and *Petalostemum* showed decreases. The addition of N altered the species responses to [CO$_2$], such that the *Amorpha* Ndfa decreased with increased [CO$_2$] under N fertilization and the other three species exhibited little or no response to elevated [CO$_2$].

Our bootstrap analysis indicated that the estimates of Ndfa based on δ^{15}N natural abundance values were sensitive to the estimates of δ^{15}N fractionation during N fixation (the B-value) when growing in ^{15}N unlabeled soils (i.e. during the ambient N treatment). However, when incorporating the uncertainty of this B-value into our model fitting, we still observed significant higher-order interactions between species

and treatments in more than 95% of the bootstrap runs (see Table 2). This suggests that the variation observed in leume leaf δ^{15}N is consistent with interactive, species-specific responses of N fixation to elevated atmospheric [CO$_2$] and N addition.

The above-ground productivity of legumes depended on species identity, atmospheric [CO$_2$], and their interaction (Fig. 3). N addition did not have a significant effect on productivity for these legume species and there were no other significant interactions (see Table 3 for ANOVA results). The variation in [CO$_2$] response was apparently caused by the much larger response of *Lespedeza* to elevated [CO$_2$] than that of the other three species.

Discussion

We observed surprising species-specific responses of legume symbiotic N fixation to elevated [CO$_2$] and N addition which were not consistent with theoretical predictions that N fixation should generally increase with increased atmospheric [CO$_2$] and decrease with increased soil N availability (Vitousek *et al.*, 2002). A number of previous studies have observed the stimulation of legume biomass production with
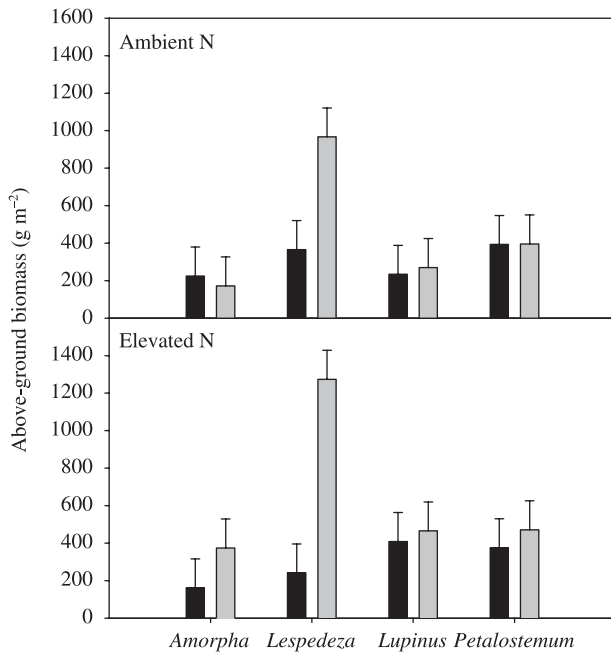
Fig. 3 Peak above-ground biomass of legumes grown at ambient and elevated atmospheric [CO_2] and N (data shown are the least-squares means + 1 standard error). Ambient [CO_2], black bars; elevated [CO_2], grey bars. Values are based on harvests from June and August and represent the production from that year only. See Table 3 for statistical analysis.

Table 3 Results of a mixed-model analysis of variance (ANOVA) of the effects of species, [CO_2] and nitrogen treatments on legume productivity

Source	F-value (degrees of freedom)	P
[CO_2]	10.29 (1,4)	0.033
N	1.47 (1,12)	0.249
[CO_2] × N	1.71 (1,12)	0.215
Species	7.02 (3,12)	0.006
[CO_2] × Species	6.02 (3,12)	0.010
N × Species	0.18 (3,12)	0.906
[CO_2] × N × Species	0.35 (3,12)	0.787

Analysis was based on the peak above-ground biomass production of four prairie legume species grown at ambient (368 μmol m^{-2} s^{-1}) and elevated (560 μmol m^{-2} s^{-1}) [CO_2] and at ambient and elevated (+4 g of N m^{-2} yr^{-1}) N.

elevated atmospheric [CO_2], especially when N is probably limiting plant growth, suggesting a stimulation of N fixation by elevated [CO_2] (Poorter & Navas, 2003). Resource-optimization models also predict stimulatory effects of elevated [CO_2] on N fixation (Vitousek & Field, 1999; Vitousek et al., 2002). However, they do not address the potential for individual legume species to vary in their responses. For example, Vitousek et al. (2002) adapted a resource-optimization model (MEL; Rastetter & Shaver,

1992) and predicted that plants should fix N under conditions where it is less costly than soil N uptake (the cost of N fixation is estimated to be ≈ 8 g of C per g of N). This model estimates N uptake costs in terms of unrealized C gain from not allocating resources to photosynthesis. The conditions that favor N fixation in this model are those that represent a high return on investment in C gain, a low return on investment in soil N acquisition, or a low cost of resources required for N fixation, and include elevated [CO_2] concentrations (high return), low soil N (low return), open plant canopies (high return), soil well exploited by roots (low return), and high availabilities of other soil resources, such as P (low cost of these resources; Vitousek et al., 2002). Several of these conditions are met in the elevated [CO_2] treatment in our experiment. The increase in N fixation observed for *Lespedeza* in response to elevated [CO_2] is consistent with model predictions and many previous experimental findings; the lack of response in the other three species is not. The degree of response across species to elevated [CO_2] appeared to be greater under ambient N conditions compared to elevated N, although the direction of response was not consistent across species. Phosphorus availability often limits symbiotic N fixation (Pearson & Vitousek, 2002; Uliassi & Ruess, 2002), and may play a role in this experiment. Although previous work has shown that P availability does not generally limit plant productivity at Cedar Creek (Tilman, 1984, 1987), legumes did increase in abundance in response to non-N nutrient addition (including P) within herbivore exclosures (Ritchie & Tilman, 1995). Interestingly, their responses were species-specific in that experiment (e.g. within exclosures *Lespedeza* actually declined upon nutrient addition). Although these interactions are beyond the scope of the current study, it remains possible that P availability limited the responses of some species to elevated [CO_2].

There are several reasons why symbiotic N fixation of legumes may respond to elevated [CO_2] and N in a species-specific manner. Variation among species in photosynthetic responses to changes in resource availability, the efficiencies of the bacterial symbionts, inherent reliance on fixed vs soil N, or differences among species in requirements for other resources, might explain interspecific variation in response to [CO_2] or N. Increased plant C uptake (photosynthesis) in response to elevated [CO_2] should simultaneously increase N demand and C supply for N fixation, resulting in the stimulation of N fixation. A lack of photosynthesis response to elevated [CO_2] could explain a lack of the N-fixation response. However, all four species in this study showed an increased rate of photosynthesis in response to elevated [CO_2] in 2002 (T. D. Lee, unpublished), suggesting species-specific variation in the photosynthetic response does not explain the observed variation in N fixation. It is worth noting that earlier in the BioCON experiment, *Lupinus* increased N fixation in response to elevated [CO_2] (Lee et al., 2003a). However, this enhancement was not repeated in the present study. It is not

clear why our results differed from that study for *Lupinus* and may indicate a temporal component to species responses to these changes (Sabo, 2003; Hungate *et al.*, 2004).

Because both the plant and bacterial genotype can affect the rates of N fixation in these symbioses (Popescu, 1998; Burdon *et al.*, 1999; Provorov & Tikhonovich, 2003), another hypothesis is that the non-responsive species lacked their ideal bacterial symbiont and therefore were maintaining a relatively inefficient symbiosis that was relatively unresponsive to environmental changes. Any change in atmospheric $[CO_2]$, and therefore potentially C supply to the rhizobia or N supply to the plant, would therefore not necessarily result in changes in N fixation. This hypothesis, however, is inconsistent with the high proportion of N obtained from fixation for all four species under ambient N conditions. It also does not explain a decline in N fixation with elevated atmospheric $[CO_2]$. As symbiotic N fixation is a mutalistic exchange between plants and bacteria, it is also possible that variation among plant species in their ability to control the outcome of the interaction (i.e. sanction rhizobia; Kiers *et al.*, 2003) could help to explain the variation we observed among species. Perhaps as conditions change (e.g. increased C or N supply), certain plant species are not able to efficiently 'sanction' poorly performing rhizobia, or 'reward' those performing well (Burdon *et al.*, 1999), resulting in a mutualism that is unresponsive to changes in resource availability.

Symbiotic N-fixation relationships can be grouped based on the morphology of the nodule formed and the compounds that are exported to the plant. *Lespedeza* has determinate 'desmodioid' nodules that export ureides, whereas the other three species have indeterminate nodules that export amides (Sprent, 2001). Although *Lespedeza* appeared to show the strongest and most predictable response to treatments, the other three species varied in their response to the treatments. Stimulation of N fixation in legumes with both indeterminate (Luscher *et al.*, 2000) and determinate (Ofosubudu *et al.*, 1995) nodules has been reported, making this dichotomy unlikely to explain the observed pattern. Our results do, however, argue for larger studies, which encompass the major variation among legumes in nodule morphology and activity, in order to understand the patterns of response to global change of symbiotic N fixation. Rapid progress is being made in understanding the evolution and phylogenies of legume species (Sprent, 2001; 2002; Doyle & Luckow, 2003), so there is great potential for future syntheses of N-fixation responses to global change in a phylogenetic and evolutionary context.

The annual addition (that started in 1998) of N, in this study, stimulated *in situ* net N mineralization in the legume plots by 39% and 71% in 2002 and 2003, respectively (data not presented), supporting our assumption that added N would increase soil N availability. The observed decline of N derived from fixation overall with this greater N supply was consistent with resource-optimization models which suggest that increased soil N availability increases the return on

investment in soil uptake relative to N fixation (Vitousek *et al.*, 2002).

With our estimates of Ndfa and N in above-ground legume biomass we can calculate the total amount of atmospherically derived N in the above-ground tissue. Although subject to certain errors (e.g. heterogeneity in N concentration among tissues), this calculation revealed similar patterns as those observed in Ndfa and tended to exaggerate the apparent advantage of *Lespedeza* (results not presented). This suggests a competitive advantage for *Lespedeza* over the other three species under conditions of elevated $[CO_2]$. This interpretation, however, does not take into account investment in below-ground productivity and the amount of N in those tissues. We presented above-ground biomass because it most clearly represents annual productivity. Good estimates of root life span for these species are lacking, and because life span can vary among species with similar growth forms (e.g. West *et al.*, 2003), below-ground biomass may not directly reflect below-ground productivity. It is worth noting that below-ground biomass differs among these species and exhibits fairly strong species-specificity in response to elevated $[CO_2]$ that does not necessarily match what is observed above ground (e.g. *Lupinus* below-ground biomass increased substantially in response to elevated $[CO_2]$; Reich *et al.*, 2001b; P. B. Reich, unpublished), suggesting complex patterns of whole-plant response to the treatments.

Although we do not yet have a detailed, mechanistic understanding of the observed species-specificity of responses, it is clear that the N-fixing symbiotic relationships between plants and bacteria will not respond to environmental change in the same manner across species. This conclusion presents an important challenge in attempts to predict ecosystem responses to environmental change, as species identity may be an important factor controlling the response of N fixation to global change.

Acknowledgements

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Appendix

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