

Stoichiometric response of nitrogen-fixing and non-fixing dicots to manipulations of CO₂, nitrogen, and diversity

Amy M. Novotny · John D. Schade · Sarah E. Hobbie ·
Adam D. Kay · Marcia Kyle · Peter B. Reich ·
James J. Elser

Received: 5 December 2005 / Accepted: 17 October 2006
© Springer-Verlag 2006

Abstract Human activities have resulted in increased nitrogen deposition and atmospheric CO₂ concentrations in the biosphere, potentially causing significant changes in many ecological processes. In addition to these ongoing perturbations of the abiotic environment, human-induced losses of biodiversity are also of major concern and may interact in important ways with biogeochemical perturbations to affect ecosystem structure and function. We have evaluated the effects of these perturbations on plant biomass stoichiometric composition (C:N:P ratios) within the framework of the BioCON experimental setup (biodiversity, CO₂, N) conducted at the Cedar Creek Natural History Area, Minnesota. Here we present data for five plant species: *Solidago rigida*, *Achillea millefolium*, *Amorpha canescens*, *Lespedeza capitata*, and *Lupinus perennis*. We found significantly higher C:N and C:P ratios under elevated CO₂ treatments, but species responded

idiosyncratically to the treatment. Nitrogen addition decreased C:N ratios, but this response was greater in the ambient CO₂ treatments than under elevated CO₂. Higher plant species diversity generally lowered both C:N and C:P ratios. Importantly, increased diversity also led to a more modest increase in the C:N ratio with elevated CO₂ levels. In addition, legumes exhibited lower C:N and higher C:P and N:P ratios than non-legumes, highlighting the effect of physiological characteristics defining plant functional types. These data suggest that atmospheric CO₂ levels, N availability, and plant species diversity interact to affect both above-ground and belowground processes by altering plant elemental composition.

Keywords BioCON · Ecological stoichiometry · Elevated CO₂ · Nitrogen enrichment · Species richness

Communicated by Russell Monson.

A. M. Novotny · M. Kyle · J. J. Elser (✉)
School of Life Sciences, Arizona State University,
Tempe, AZ 85287-4501, USA
e-mail: j.elser@asu.edu

J. D. Schade
Environmental Studies, St Olaf College,
Northfield, MN 55057, USA

S. E. Hobbie · P. B. Reich
Department of Ecology, Evolution, and Behavior,
University of Minnesota, St Paul, MN 55108, USA

A. D. Kay
Department of Biology,
University of St Thomas, St Paul, MN 55105, USA

Introduction

Considerable research has documented a variety of global environmental changes resulting from human activities. Of particular interest are the steady increase in atmospheric carbon dioxide (CO₂) concentration over the past two centuries (Vitousek 1994), increases in nitrogen (N) inputs to ecosystems from fertilization and atmospheric deposition (Sala et al. 2000; Norby 1998), and the rapid loss of biodiversity (Sala et al. 2000). The simultaneous occurrence of these global changes raises questions about how their interactions will influence ecosystems and individual species in the coming decades.

Increasing atmospheric CO₂ concentrations have been shown to affect many ecosystem processes. In

particular, plant growth rate and net biomass accumulation increase under elevated CO₂ concentrations in many ecosystems (Niklaus et al. 1998; King et al. 2004; Torbert et al. 2004), although this response may only be observed after several years of CO₂ enrichment (Winkler and Herbst 2004). The extent and nature of ecosystem response to elevated CO₂ may be influenced by N availability, with ecosystems with low N showing a more modest response to elevated CO₂ than those with high N (Cannell and Thornley 1998). Since productivity in many terrestrial and aquatic ecosystems is limited by N (Elser et al. 1990; Vitousek and Howarth 1991), changes in CO₂ and N must be considered together to understand their effects on plant communities and ecosystem processes. Local biodiversity and plant functional type (e.g., N-fixing legumes vs. non-leguminous forbs) may also be involved in mediating how CO₂ and N perturbations affect ecosystems. For example, studies have shown that enhancement of atmospheric CO₂ and N deposition separately leads to increased biomass production but that this response is lower in species-poor areas (Reich et al. 2001b). In addition, legumes tend to be more productive than non-N fixing forbs under elevated CO₂ conditions because of the stimulation of N₂ fixation (Lüscher et al. 1998; Reich et al. 2001b). These studies highlight the interactive roles that biodiversity, plant functional type, and species identity play in ecosystem-level responses to changes in CO₂ and N deposition.

Because global environmental changes clearly influence multiple plant physiological processes involved with the acquisition of energy and nutrients, we would expect to see a corresponding change in the ratios of major elements [i.e., C, N, and phosphorus (P)] in plant tissue (Marschner 1995; Aerts and Chapin 2000; Sterner and Elser 2002). These stoichiometric ratios are important from a functional perspective as indicators of substrate quality for herbivores (Elser et al. 2000) and decomposers (King et al. 2004), which in turn influences the fate of the production of organic matter (Cebrian 1999). For these reasons, it is important to understand how changes in CO₂, N, and biodiversity jointly affect this aspect of plant response. Elevated CO₂ treatments often lead to decreases in the percentage of N (%N) or increases in the C:N ratio in plant leaf tissue in a variety of species (Gifford et al. 2000; Reich et al. 2001a; Billings et al. 2003; King et al. 2004). Plant functional type also tends to modify the way C:N ratios respond to elevated CO₂, as legumes may show less increase in C:N ratios (due to simultaneous increases in atmospheric N₂ fixation) than non-legumes (Torbert et al. 2004). Elevated N deposition has also been shown to reduce plant C:N ratios in a

variety of ecosystems (Norby 1998; Rueth and Baron 2002).

While C:N ratios are commonly used indicators of physiological responses to elevated CO₂, other potential stoichiometric changes are relatively unstudied. For example, few studies have examined how increased CO₂ affects plant biomass N:P or C:P ratios. Of these, one by Gifford et al. (2000) showed that elevated CO₂ levels induce an increase in C:P ratios, while that of Niklaus et al. (1998) found no change in the N:P ratios. Furthermore, the interactive effects of CO₂ and N on plant C:P and N:P ratios and the role of biodiversity and plant functional type in modifying their effects remain to be studied. A focus on C:P and N:P ratios is important because of the previously unappreciated role of P intake as an aspect of food quality for herbivores (Elser et al. 2000; Perkins et al. 2004) and because P can be a critical limiting nutrient for microbial processes, even if plant production is limited by other factors (Sundareshwar et al. 2003).

In the study reported here, we examined changes in foliar stoichiometric ratios under manipulations of CO₂ levels, N addition, and plant species diversity (richness). We sampled five commonly found dicot plant species in a grassland ecosystem in Minnesota where plants are grown experimentally in a full factorial combination of CO₂, N addition, and diversity levels. Because some studies show functional type differences in C:N response to elevated CO₂ and nitrogen, we selected species that were representative of both N₂-fixing legumes and non-N₂ fixing forbs. Our results indicate a variety of direct and interactive effects of CO₂, N, and biodiversity on plant C:N:P stoichiometry and have significant potential implications for terrestrial ecosystems and food webs.

Material and methods

Experimental design

This study was conducted as part of the BioCON (Biodiversity, CO₂, and N) experimental setup performed at the Cedar Creek Natural History Area in Minnesota, USA (Reich et al. 2001a, b). The design consists of six 20-m diameter rings distributed across a grassland field, with each ring containing sets of 2×2-m plots representing fully crossed treatments of species diversity (1, 4, 9, 16 species), and N addition (no addition vs. +4 g N m⁻² year⁻¹). Three rings use a free air CO₂ enrichment system (FACE) that supplies air with an elevated CO₂ concentration (approx. 560 ppm) to the plots, while three control plots receive ambient levels

of CO₂ (approx. 370 ppm). One-half of the plots within the rings were selected randomly to receive N fertilizer three times each year for a total N addition of 4 g N m⁻² year⁻¹ as NH₄NO₃. Each treatment combination was replicated at least two times within a ring. Species selected for this experiment were native to neighboring prairie and grassland areas or have been naturalized to the area (Reich et al. 2001a, b). Additional details and background information about BioCON and the experimental setup can be found in Reich et al. (2001a, b).

While plots within the main BioCON experiment contained up to 16 species, only monocultures and four-species plots were used in this study because of time constraints. During the second week of July 2002, we collected leaf samples from five plant species: two non-legume forbs (*Solidago rigida* and *Achillea millefolium*) and three legumes (*Amorpha canescens*, *Lespedeza capitata*, and *Lupinus perennis*). We sampled from monocultures and from four-species plots that represented one functional type, either legume or non-legume forb. This allowed us to examine the effects of species diversity while holding functional group diversity constant (as in Reich et al. 2004). Shoots from five plants were sampled from each of at least two replicate plots from each of the CO₂ and N treatment combinations. Samples were dried at 60°C for 72 h, then milled and analyzed for C, N, and P content. Carbon and N analyses were completed on a PE-2400 CHN analyzer (Perkin-Elmer, Foster City, Calif.) at Arizona State University. Leaf P content was measured by persulfate oxidation followed by colorimetric analysis (Schade et al. 2003). All stoichiometric ratios (C:N, C:P, N:P) are reported as molar ratios. Ratios were averaged per plot prior to statistical analyses.

Statistical analysis

An analysis of variance (ANOVA) was performed on the data since the assumptions of normality and equal variance were upheld based on examination of plots of the expected value for normal distribution versus residuals. The main effects of diversity, CO₂, and N manipulations along with functional type and species (nested in functional type) and their interactions were tested for each stoichiometric ratio. Because this was a split-plot design, the “ring” was tested as a random effect nested within the CO₂ treatment. Significant differences among treatment means were then analyzed using the post-hoc Tukey’s multiple comparison test. Least squares means plots are shown only for significant effects and interactions. Note that the scale of the BioCON experiment implies that the

level of replication of the treatment combinations ($n=2$) and the number of treatment levels [CO₂ and N enrichment have only two levels (enriched or control) and in our analysis, only two diversity levels are considered] are limited. Thus, our analysis may be unable to detect all actual effects. Nevertheless, various manipulations resulted in statistically significant differences among the treatments, especially for the C:N ratio, and we confine our discussion and interpretation to these patterns.

Results

Foliar C:N:P ratios of the five plant species considered in our study responded significantly to manipulations of CO₂ and N supply. However, these responses depended on local species diversity and the identity or functional type of the species involved. In the following sections we summarize the main effects followed by the interactions for each of the dependent variables examined in the study.

C:N ratios

The C:N ratio (hereafter, C:N) responded significantly to all of the independent factors tested in this experiment: CO₂ and N enrichment, species identity, diversity, and plant functional type. The five species differed significantly in C:N on average across all treatment variables ($P<0.001$; Fig. 1a; Table 1). The range in C:N was substantial, with *Lupinus* and *Amorpha* averaging as low as approximately 21 and *Solidago* averaging approximately 33. Despite these chemical differences at the species level, on average across all five species, CO₂ enrichment produced a significant increase in the C:N ($P<0.001$; Fig. 1b), with C:N increasing from approximately 24 to approximately 28 under enriched CO₂. Nitrogen addition caused a statistically significant decline in C:N ($P=0.036$; Fig. 1c; Table 1); however, the absolute magnitude of this effect was modest (C:N decreased from 26.6 to 25.6 under conditions of enriched N). Increased local plant diversity also resulted in lower C:N ($P<0.001$; Fig. 1d; Table 1). Furthermore, functional type had a significant effect, as non-leguminous forbs had a significantly higher C:N than legumes (approx. 29.4 for non-legumes compared to approx. 22.7 for legumes) ($P<0.001$; Table 1; see Fig. 1a).

For each of the manipulated factors there were multiple significant interactions that also contributed to C:N response. The effect of CO₂ enhancement interacted with plant species identity ($P=0.001$; Table 1), as only three species (*Lespedeza*, *Achillea*, and *Solidago*)

Fig. 1 Least squares means plots of the main effects of species identity (a), of CO₂ (b), of N (c), and of diversity (d) on plant C:N ratios. Effects of plant functional type can be seen by inspection of a, as *Amorpha*, *Lespedeza*, and *Lupinus* are N-fixing legumes (left-hand side of a), while *Achillea* and *Solidago* are non-fixing forbs (right hand side of a). Letters above each column differ when the column means are significantly different from each other according to a Tukey's multiple comparisons test. Error bars indicate ± 1 SE

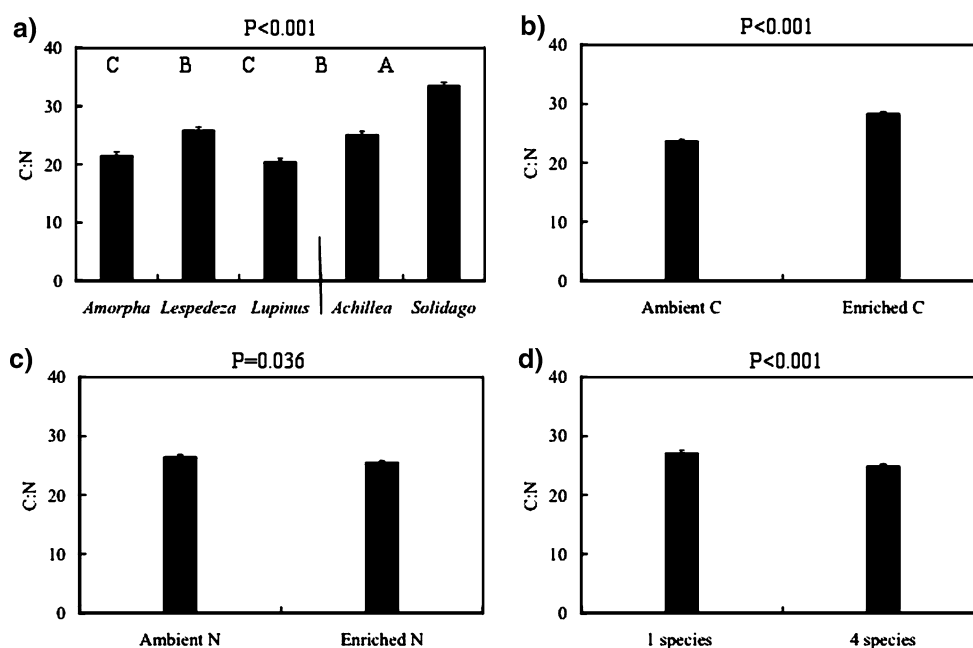


Table 1 Results of analysis of variance (ANOVA) for plant C:N, C:P, and N:P ratios indicating main effects and interactions of carbon (CO₂ of approx. 360 ppm vs. approx. 550 ppm), diversity (mono-specific vs. four species), N (no addition vs. +4 g N m⁻²

year⁻¹), plant functional type (legume vs. forb), and species (*Solidago rigida*, *Achillea millefolium*, *Amorpha canescens*, *Lespedeza capitata*, and *Lupinus perennis*)

Source	df	C:N		C:P		N:P	
		F ratio	P value ^a	F ratio	P value ^a	F ratio	P value ^a
Carbon	1	129.3	**	2.12	–	0.99	–
Ring[Carbon] and Random	4	0.807	–	5.94	**	3.9	**
Diversity	1	21.9	***	9.67	**	0.074	–
Diversity × carbon	1	8.38	**	0.301	–	1.011	–
Nitrogen	1	4.64	*	0.073	–	0.587	–
Nitrogen × diversity	1	1.22	–	0	–	0.53	–
Nitrogen × carbon	1	6.31	*	0.241	–	0.46	–
Nitrogen × carbon × diversity	1	0.623	–	0.637	–	0.21	–
Type	1	212.2	***	511	***	712	***
Type × diversity	1	4.87	*	0.485	–	0.23	–
Type × carbon	1	17	***	0.376	–	0.018	–
Type × diversity × carbon	1	9.15	**	1.27	–	3.24	(*)
Type × nitrogen	1	2.23	–	0.055	–	0.434	–
Type × nitrogen × diversity	1	0.081	–	1.16	–	1.16	–
Type × nitrogen × Carbon	1	3.07	(*)	0.932	–	1.67	–
Type × nitrogen × carbon × diversity	1	0.114	–	1.52	–	1.53	–
Species [Type]	3	68	***	30.3	***	48.3	***
Species × carbon[Type]	3	6.04	**	5.81	**	2.17	–
Species × nitrogen[Type]	3	0.157	–	0.044	–	0.034	–
Species × diversity[Type]	3	1.64	–	8.81	***	5.71	**
Species × carbon × nitrogen[Type]	3	1.83	–	2.21	(*)	0.973	–
Species × carbon × diversity[Type]	3	7.77	–	0.879	–	0.666	–
Species × nitrogen × diversity[Type]	3	18.3	–	0.468	–	0.213	–
Species × nitrogen × diversity × carbon[Type]	3	7.41	–	1.81	–	0.826	–

^a P values are represented by the following symbols: –, Non-significant; (*), 0.10 > P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001

had significant increases in C:N under elevated CO₂ (Fig. 2a). Thus, plant C:N response to CO₂ was somewhat idiosyncratic among the taxa. Nitrogen addition had a significant negative effect on C:N under ambient

CO₂ levels, but no effect on C:N under elevated CO₂ (significant CO₂-N interaction, P=0.015; Fig. 2b; Table 1). However, there was no significant N-species interaction (P>0.05; Table 1).

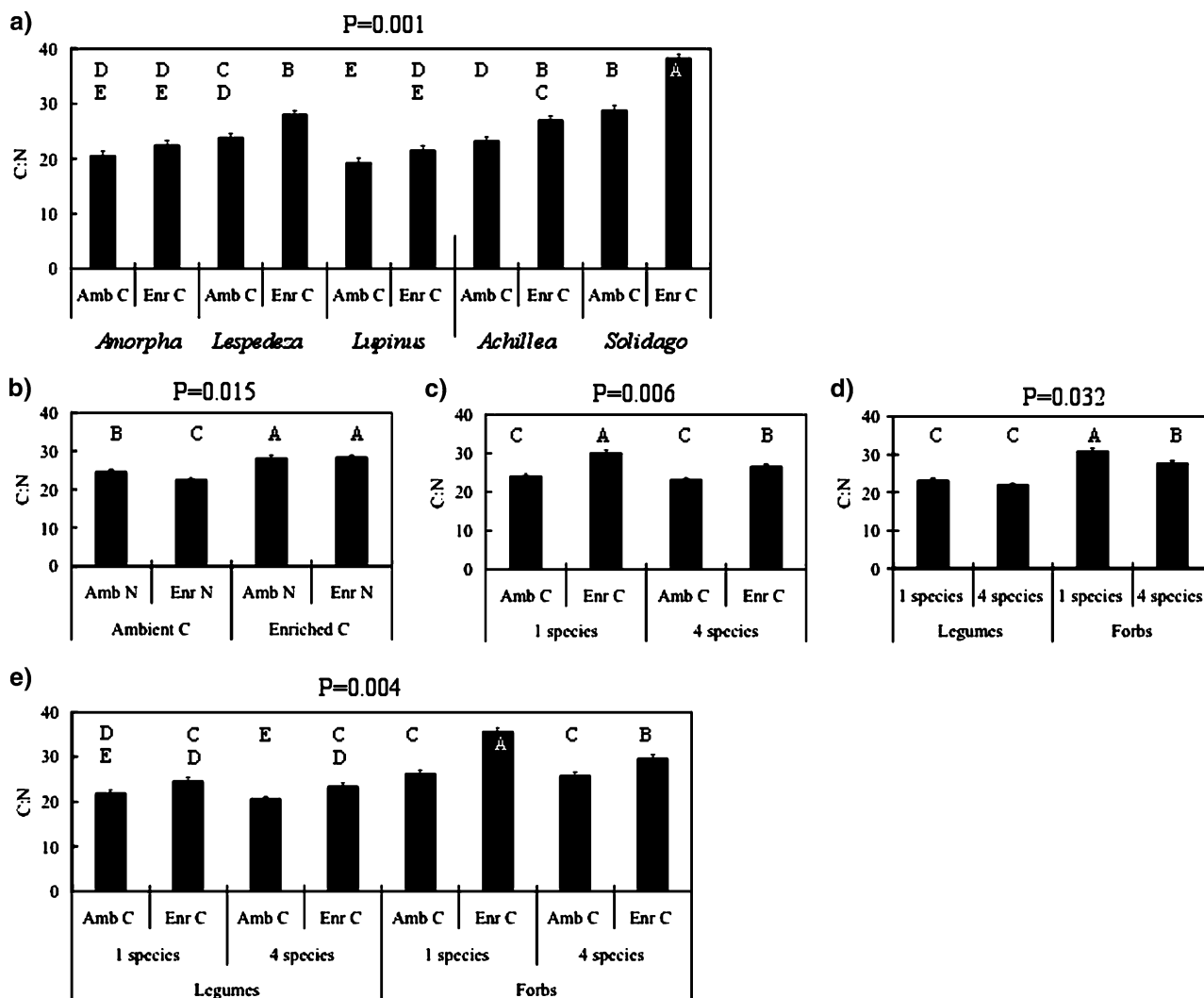


Fig. 2 a–d Least squares means plots of the two-way interactions of CO₂ (Amb C ambient CO₂, Enr C enriched CO₂) and species (a), of CO₂ and N (Amb N ambient N, Enr N enriched N) (b), of CO₂ and diversity (1 sp monospecific plot, 4 spp four-species plot) (c), and of diversity and functional type (Legume N-fixer, Forbs non-N-fixer) (d) on C:N ratios. The two-way interaction between CO₂ and functional type on the C:N ratio can be seen

following an inspection of a as *Amorpha*, *Lespedeza*, and *Lupinus* are N-fixing legumes (left-hand side of a), while *Achillea* and *Solidago* are non-fixing forbs (right-hand side of a). (e) Least squares means plot of the three-way interaction of CO₂, diversity, and type on C:N ratios. Letters above each column differ when the column means are significantly different from each other according to a Tukey’s multiple comparisons test. Error bars indicate ±1 SE

Diversity also affected how C:N responded to CO₂ enrichment. Under low diversity, CO₂ enrichment led to a larger increase in plant C:N than it did under high diversity ($P=0.006$; Table 1; Fig. 2c). The interactive effect of diversity and N on the C:N ratio was not significant ($P>0.05$; Table 1). However, the effect of diversity did depend on plant functional type, as non-legumes had a larger decrease in C:N with increased diversity than did legumes (Fig. 2d), leading to a significant diversity-type interaction ($P=0.032$; Table 1).

C:N also responded differently to CO₂ addition in a plant functional-type manner. While plants of both functional types had higher C:N with CO₂ addition (see Fig. 2a), the response of the non-leguminous forbs was

greater (significant CO₂-type interaction; $P<0.001$; Table 1). However, there was no statistical interaction between N enrichment and functional type on the plant C:N ratio ($P>0.05$; Table 1).

Finally, plant functional type appeared to modulate the response of plant C:N to CO₂ and diversity, as indicated by a significant three-way CO₂-diversity-type interaction ($P=0.004$; Table 1; Fig. 2e). Both legumes and non-legumes responded to CO₂ enrichment with an increase in C:N under both low and high diversity. While the magnitude of the increase was the same under both diversity levels for the legumes, in the non-legumes C:N increased more in response to CO₂ in the low diversity treatment than in the high diversity treatment (Table 1;

Fig. 2e). There were no other significant three-way interactions ($P>0.05$; Table 1).

C:P ratios

All five species in our study had significantly different C:P ratios (hereafter, C:P) on average across all treatments ($P<0.001$; Table 1), covering a twofold range (approx. 357 for non-leguminous *Achillea* to approx. 860 for the leguminous *Lupinus*; Fig. 3a). Carbon dioxide enrichment and N manipulation had no significant effects on C:P (Table 1). However, C:P responded to local plant diversity as well as plant functional type. Overall, plant C:P, like C:N, decreased significantly

from approximately 613 in the monocultures to approximately 563 in the four-species plots ($P=0.003$; Table 1; Fig. 3b). Furthermore, legumes had significantly higher C:P than the non-legumes (approx. 763 vs. approx. 412) when species were pooled across all treatments ($P<0.001$; Table 1; see Fig. 3a).

The C:P was affected by several interactions between factors. While there was no significant CO_2 -N interaction for C:P, there was a significant species- CO_2 interaction ($P=0.002$; Table 1), which was driven largely by the strong increase in *Lespedeza* C:P in response to CO_2 enrichment (Fig. 3c). The species-diversity interaction was also significant ($P<0.001$; Fig. 3d; Table 1) as three species (*Lespedeza*, *Lupinus*, and

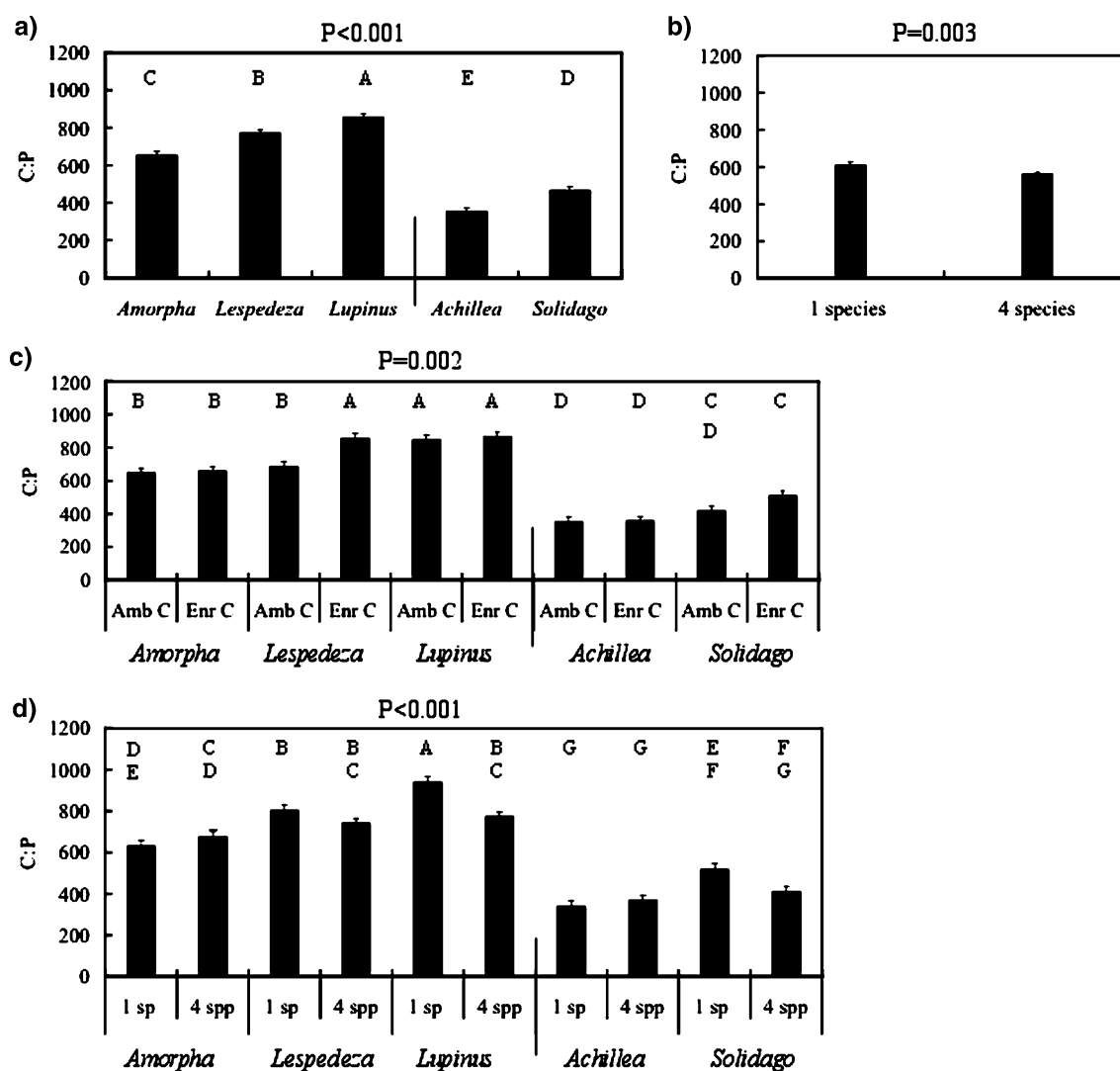


Fig. 3 a, b Least squares means plots of the main effects of species identity (a) and of diversity (b) on C:P ratios. As in Fig. 1, effects of plant functional type can be seen by inspection of a, as *Amorpha*, *Lespedeza*, and *Lupinus* are N-fixing legumes (left-hand side of a) while *Achillea* and *Solidago* are non-fixing forbs (right-hand side of a). (c, d) Least squares means plots of the

two-way interactions of species and CO_2 (Amb C ambient CO_2 , Enr C enriched CO_2) (c) and of species and diversity (1 sp monospecific plot, 4 spp four-species plot) (d) on C:P ratios. Letters above each column differ when the column means are significantly different from each other according to a Tukey's multiple comparisons test. Error bars indicate ± 1 SE

Solidago) had C:P ratios that decreased under high diversity (only that of *Lupinus* was significant), while the C:P ratios of *Amorpha* and *Achillea* increased slightly (but not significantly) under high diversity (Table 1; Fig. 3b). No other interactions significantly influenced C:P (Table 1).

N:P ratios

Species differed significantly in N:P ratios (N:P) on average across all treatments ($P < 0.001$; Table 1), exhibiting a threefold range in N:P across the species from approximately 14 (*Achillea* and *Solidago*) to approximately 42 (*Lupinus*). However, not all species were significantly different from each other (Fig. 4a). The N:P of *Lupinus* was significantly greater than those of the remaining four species. *Amorpha* and *Lespedeza* had higher N:P than the non-legumes, but the

ratios of *Amorpha* and *Lespedeza* were not significantly different from each other. Likewise, *Achillea* and *Solidago* N:P were significantly lower than those of the legumes but not significantly different from each other (Fig. 4a). None of the primary independent variables (CO_2 or N manipulations, diversity) had a significant main effect on the N:P of these five species (Table 1). However, legumes did have significantly higher N:P (approx. 34) than non-leguminous forbs (approx. 14), resulting in a significant functional type effect ($P < 0.001$; Table 1; see Fig. 4a).

CO_2 and N manipulations had no interactive effects on N:P (Table 1), but the species-diversity interaction was significant ($P = 0.002$; Table 1; Fig. 4b), indicating that the response of N:P to diversity manipulation depended on the identity of the species. *Lespedeza*, *Lupinus*, and *Solidago* all had lower N:P with increasing diversity, while *Amorpha* and *Achillea* had higher N:P when diversity was high. However, none of the diversity effects within a species were statistically significant (Fig. 4b). No other interaction terms were statistically significant for N:P (Table 1).

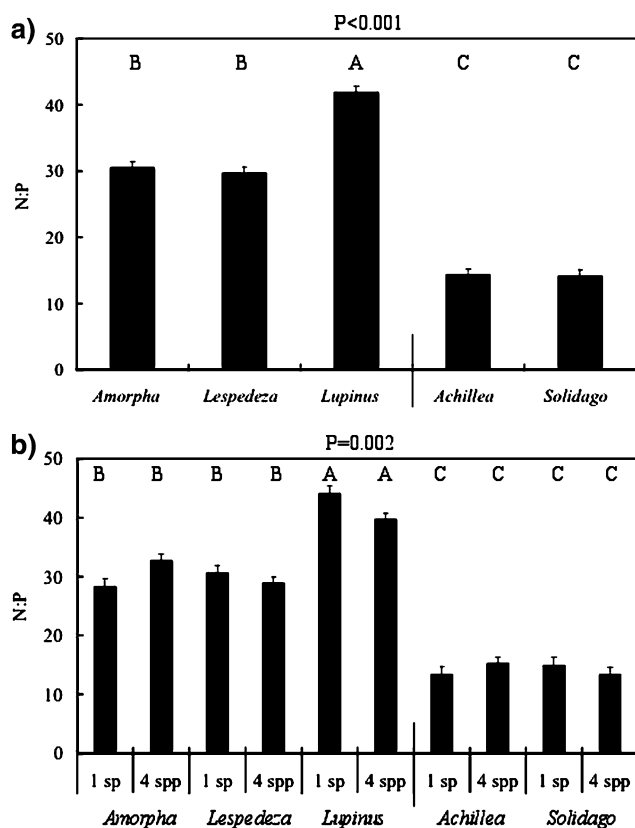


Fig. 4 Least squares means plot of the main effect of species (a) on N:P ratios. As in Fig. 1, effects of plant functional type can be seen by noting that *Amorpha*, *Lespedeza*, and *Lupinus* are N-fixing legumes (left-hand side) while *Achillea* and *Solidago* are non-fixing forbs (right-hand side). Least squares means plot of the two-way interaction of species and diversity (1 sp monospecific plot, 4 spp four-species plot) (b) on N:P ratios. Letters above each column differ when the column means are significantly different from each other according to a Tukey's multiple comparisons test. Error bars indicate ± 1 SE

Discussion

Our data show that foliar stoichiometric ratios responded to changes in CO_2 , N, and diversity despite overall differences in tissue chemistry among species. Enriched CO_2 treatments alone affected elemental ratios, but these responses to elevated CO_2 were also mediated by N and diversity treatments. Plant functional type also influenced how stoichiometric ratios responded to elevated CO_2 . It is important to note that our data pertain only to foliar nutrient contents on a single date during the growing season and thus may not ramify to ecosystem-level nutrient cycling effects such as nutrient use efficiency (Vitousek 1982) if observed ratios are not correlated with overall plant nutrient use during the growing season or with the nutrient content of senesced tissue (Killingbeck 1996). In general, foliar nutrient content correlates closely with nutrient content in other plant tissues (Kerkhoff et al. 2006), and thus foliar data are relevant to whole-plant nutrient use. Furthermore, foliar nutrient content is positively correlated with that of senesced leaves (Kobe et al. 2005). Consequently, our data set, while somewhat limited, should nevertheless have relevance in gaining an understanding of how plant physiological response to CO_2 , nitrogen, and diversity may affect nutrient cycling processes.

The effects of elevated CO_2 on plant stoichiometric (C:N:P) ratios depended on both species identity and N

fertilization levels. Of the five species investigated, the average increase in C:N in response to elevated CO₂ was similar to increases found in other studies (Niklaus et al. 1998; Gifford et al. 2000; Reich et al. 2001a), but the magnitude of this response in our study differed among species. Three of the five species, *Lespedeza*, *Achillea*, and *Solidago*, responded with substantial increases in C:N (Fig. 2a). Similarly, in terms of changes in C:P, species responded idiosyncratically to elevated CO₂, with the only significant increase in C:P observed in *Lespedeza* (Fig. 3c). Stoichiometric response to elevated CO₂, however, also depended on N enrichment. While there was an overall increase in C:N in response to CO₂ enrichment, this CO₂ response was larger under enriched N (Fig. 2b). Furthermore, N addition resulted in lower C:N, but only under ambient CO₂ (Fig. 2b). This contrasts with the findings of Gifford et al. (2000) who showed a stronger C:N response to elevated CO₂ under low levels of N deposition; however, that study focused on only a single species. Importantly, our data demonstrate over multiple plant species that atmospheric changes in CO₂ and N deposition can affect C:N:P stoichiometry directly but also that these changes have inter-dependent effects.

While stoichiometric responses to CO₂ and nutrient manipulations have been examined previously, a more unique, and potentially important, outcome of our study is the documentation that biodiversity modifies plant stoichiometric response to CO₂ (Fig. 2c). In general, elevated levels of diversity in terms of species richness lowered both C:N and C:P (Figs. 1d, 3b), but diversity also significantly affected how plants responded to elevated CO₂. In the monocultures, increasing CO₂ led to an amplified increase in C:N relative to the four-species plots (Fig. 2c). A possible explanation for this difference is that, in monocultures, individuals likely experience a more severe competition for nutrients due to an increased overlap of resource use strategies with conspecific neighbors. However, in high diversity plots, neighbors will often be of a different species, which could reduce potential resource use overlap and increase nutrient availability for individual plants. This hypothesis could link these stoichiometric responses to diversity to the findings of Reich et al. (2001a), who argued that overall biomass increases due to elevated CO₂ or N were higher in species-rich than species-poor treatments because of inter-specific niche partitioning. Furthermore, such a variation in the severity of intra- and interspecific competition may underlie the species-specific variation in C:P and N:P response to diversity that we observed.

Plant functional types also differed in stoichiometric ratios and in their response to elevated CO₂. Legumes

generally had lower C:N (Fig. 1a), higher C:P (Fig. 3a), and higher N:P (Fig. 4a) than non-legumes, thereby reflecting that legumes are more likely to be P-limited than non-legumes. In response to elevated CO₂, however, C:N increased more in non-legumes than in legumes (Fig. 2a), presumably because N₂ fixation by legumes allows them to match enhanced C assimilation due to increased CO₂ concentration with additional N drawn from N fixation (see also Reich et al. 2001a,b; Lee et al. 2003; Torbert et al. 2004).

Our data show that foliar stoichiometric ratios are impacted by changes in CO₂, N, and plant diversity. Such changes are relevant ecologically because plant elemental composition potentially affects important ecosystem processes and ecological interactions, such as ecosystem nutrient use efficiency (Vitousek 1982), litter decomposition, and herbivore performance. Previous studies have shown that the nutrient content of decomposing biomass can influence decomposition rates (Enriquez et al. 1993). Thus, changes in plant stoichiometric ratios due to CO₂, N, or diversity changes could also imply changes in litter decomposition rates, if litter and plant elemental composition are functionally correlated (Hobbie 1992). In our study, elevated CO₂ led to higher plant C:N ratios, possibly leading to slower decomposition rates, as observed in some studies (De Angelis et al. 2000; Liao et al. 2002; Schadler et al. 2003) but not in others (Norby et al. 2001; Dukes and Hungate 2002). For species that do experience such changes in elemental composition, this stoichiometric shift could buffer global CO₂ increases, as plant nitrogen-use efficiency (NUE) increases, while slower decomposition rates lead to an increase in the pool size of soil C. Importantly, diversity modified these responses. High diversity significantly dampened the increase of biomass C:N in response to elevated CO₂, indicating that high diversity will tend to offset potential stoichiometric effects on C storage in plant standing stocks and soils. Our data also have implications for the possible impacts of altered CO₂, nutrients, and diversity on terrestrial food webs, since an important aspect of the quality of plant biomass for herbivores is its nutrient (N, P) content (White 1993; Elser et al. 2000). Previous studies have shown that growing plants under elevated CO₂ levels can impair herbivore performance (Stiling et al. 2003; Hamilton et al. 2004). Our data add to these findings in two ways. First, the moderating effect of plant diversity on plant C:N increase in response to atmospheric CO₂ suggests that maintenance of plant diversity may be important in maintaining the production of higher trophic levels under conditions of increasing CO₂. Secondly, P availability can limit herbivore growth in nature (Elser et al. 2000;

Schade et al. 2003; Perkins et al. 2004), an effect that is more likely when plant N:P exceeds the N:P of herbivore biomass (Sterner and Elser 2002). In our study, the combination of increased CO₂ and N inputs along with low diversity tended to increase plant biomass C:P and N:P. In such a situation, we predict an increased prevalence of herbivore P-limitation, especially for P-rich herbivore taxa (Sterner and Elser 2002), with further potential impacts on detrital processing of organic matter as unconsumed production enters detrital pathways and affects long-term storage pools (Cebrian 1999).

Conclusion

Given the multivariate global environmental changes that are occurring, we should expect stoichiometric ratios of plant biomass to respond as well. The results of our study suggest that there are significant interactions between CO₂, N, and diversity that modify how plant elemental ratios respond to important environmental drivers. Importantly, differences in local biodiversity may affect ecosystem adjustments to biogeochemical perturbations, such as increased CO₂ or N deposition. Given that plant elemental composition responds to various environmental changes, a stoichiometric perspective may help elucidate some of the mechanisms by which global environmental changes will affect plant physiology and plant-mediated above- and belowground processes. While the immediate effects of global change may be easily seen on the plant level, higher trophic levels may also be affected via indirect mechanisms such as those we document here.

Acknowledgements Support for this research was provided by the US National Science Foundation (IRCEB program, DEB-9977047; LTER program, DEB-0080382; Biocomplexity program; DEB-0322057) and the U.S. Department of Energy (FG02-96ER62291). Special thanks are extended to the following individuals who have helped on this project: William Fagan, John Sabo, David Lewis, Dan Hernandez, and Megan Ogdahl. The experiments conducted in this project comply with the current laws of the United States.

References

Aerts R, Chapin FS (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv Ecol Res* 30:1–67

Billings SA et al (2003) Effects of elevated carbon dioxide on green leaf tissue and leaf litter quality in an intact Mojave Desert ecosystem. *Global Change Biol* 9:729–735

Cannell M, Thornley J (1998) N-poor ecosystems may respond more to elevated [CO₂] than N-rich ones in the long term. A model analysis of grassland. *Global Change Biol* 4:431–442

Cebrian J (1999) Patterns in the fate of production in plant communities. *Am Nat* 154:449–468

De Angelis P, Chigwerewe KS, Mugnozza GES (2000) Litter quality and decomposition in a CO₂-enriched Mediterranean forest ecosystem. *Plant Soil* 224:31–41

Dukes JS, Hungate BA (2002) Elevated carbon dioxide and litter decomposition in California annual grasslands: which mechanisms matter? *Ecosystems* 5:171–183

Elser JJ, Sterner RW, Gorokhova E, Fagan WF et al. (2000) Biological stoichiometry from genes to ecosystems. *Ecol Lett* 3:540–550

Elser JJ, Marzolf ER, Goldman CR (1990) Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. *Can J Fish Aquat Sci* 47:1468–1477

Enriquez S et al. (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. *Oecologia* 94:457–471

Gifford RM, Barrett DJ, Lutze JL (2000) The effects of elevated [CO₂] on the C:N and C:P mass ratios of plant tissues. *Plant Soil* 224:1–4

Hamilton JG, Zangerl AR, Berenbaum MR, Phippen J et al. (2004) Insect herbivory in an intact forest understory under experimental CO₂ enrichment. *Oecologia* 138:566–573

Hobbie SE (1992) Effects of plant species on nutrient cycling. *Tree* 7:336–339

Kerkhoff A et al. (2006) Phylogenetic and functional variation in the scaling of nitrogen and phosphorus in the seed plants. *Am Nat* 168:E103–E122

Killingbeck KT (1996) Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. *Ecology* 77:1716–1727

King JY et al. (2004) Plant nitrogen dynamics in shortgrass steppe under elevated atmospheric carbon dioxide. *Ecosystems* 7:147–160

Kobe RK, Lepczyk CA, Iyer M (2005) Resorption efficiency decreases with increasing green leaf nutrients in a global data set. *Ecology* 86:2780–2792

Lee TA et al. (2003) Contrasting growth response of an N₂-fixing and non-fixing forb to elevated CO₂: dependence on soil N supply. *Plant Soil* 255:475–486

Liao JX, Hou ZD, Wang GX (2002) Effects of elevated CO₂ and drought on chemical composition and decomposition of spring wheat (*Triticum aestivum*). *Funct Plant Biol* 29:891–897

Lüscher A, Hendrey GR, Nösberger J (1998) Long-term responsiveness to free air CO₂ enrichment of functional types, species and genotypes of plants from fertile permanent grassland. *Oecologia* 113:37–45

Marschner H (1995) Mineral nutrition of higher plants. Academic, London

Niklaus PA, Spinnler D, Körner Ch (1998) Nutrient relations in calcareous grassland under elevated CO₂. *Oecologia* 116:67–75

Norby RJ (1998) Nitrogen deposition: a component of global change analyses. *New Phytol* 139:189–200

Norby RJ, Cotrufo MF, Ineson P et al. (2001) Elevated CO₂, litter chemistry, and decomposition: a synthesis. *Oecologia* 127:153–165

Perkins MC, Woods HA, Harrison JF, Elser JJ (2004) Dietary phosphorus affects the growth of larval *Manduca sexta*. *Arch Insect Biochem Physiol* 55:153–168

Reich PB, Knops T, Tilman D et al. (2001a) Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO₂ and N deposition regimes? A field test with 16 grassland species. *New Phytol* 150:435–448

- Reich PB, Tilman D, Craine J et al. (2001b) Plant diversity enhances ecosystem responses to elevated CO₂ and nitrogen deposition. *Nature* 410:809–812
- Reich PB, Tilman D, Naeem S, Ellsworth DS et al. (2004) Species and functional group diversity independently influence biomass accumulation and its response to CO₂ and N. *Proc Natl Acad Sci USA* 101:10101–10106
- Rueth HM, Baron JS (2002) Differences in Englemann spruce forest biogeochemistry east and west of the Continental Divide in Colorado, USA. *Ecosystems* 5:45–57
- Sala OE et al. (2000) Global biodiversity scenarios for the year 2100. *Science* 287:1770
- Schade JD et al. (2003) Stoichiometric tracking of soil nutrients by a desert insect herbivore. *Ecol Lett* 6:96–101
- Schadler M et al. (2003) Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. *Oikos* 103:121–132
- Sterner RW, Elser JJ (2002) *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton, New Jersey
- Stiling P et al. (2003) Elevated CO₂ lowers relative and absolute herbivore density across all species of a scrub-oak forest. *Oecologia* 134:82–87
- Sundareshwar PV, Morris JT, Koepfler EK, Fornwalt B (2003) Phosphorus limitation of coastal ecosystem processes. *Science* 299:563–565
- Torbert HA, Prior SA, Rogers HH et al. (2004) Elevated atmospheric CO₂ effects on N fertilization in grain sorghum and soybean. *Field Crops Res* 88:57–67
- Vitousek PM (1982) Nutrient cycling and nutrient use efficiency. *Am Nat* 119:553–572
- Vitousek PM (1994) Beyond global warming: ecology and global change. *Ecology* 75:1861–1876
- Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13:87–115
- White TCR (1993) *The inadequate environment: nitrogen and the abundance of animals*. Springer, Berlin Heidelberg New York
- Winker JB, Herbst M (2004) Do plants of a semi-natural grassland community benefit from long-term CO₂ enrichment? *Basic Appl Ecol* 5:131–143